TO STUDY THE PHASE TRANSITION SYSTEM BY THE ADDITION OF CHITOSAN VIA NASAL ROUTE.

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IN

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CERTIFICATE

This is to certify that this dissertation entitled **"To Study the Phase Transition System By the Addition of Chitosan Via Nasal Route"** is a bonafide and genuine research work carried out by **Mr. Amol B. Garde (Reg.No.26081301)** in partial fulfillment of the requirements for the **Degree of Master of Pharmacy** in **Pharmaceutics**, of The Tamilnadu Dr. M .G. R. Medical University, in the Red Cross Formulation,Aurangabad (Maharashtra) under the guidance of **Mr.T.Akelesh**, Assistant Professor Department of Pharmaceutics R.V.S. College Pharmaceutical Sciences,Sulur, Coimbatore and **Mr.N.P.Kulkarni, Plant Head** under our supervision and guidance.

This dissertation is now ready for examination.

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DECLARATION

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List of Abbreviations

GIT	Gastro intestinal tract
CNS	Central nervous system
CSF	Cerebro spinal fluid
TEER	Trans epithelial electrical resistance
BBB	Blood brain barrier
HPBCD	Hydroxy propyl beta cyclodextrin
EDTA	Ethylene diamine tetra acetic acid
HCL	Hydro chloric acid
IR	Infra red
FTIR	Fourier transform infra red
UV	Ultra violet
SD	Standard deviation
nm	Nano meter
PBS	Phosphate buffer saline
TJ	Tight junction
I.P.	Indian pharmacopeia
USP	United state pharmacopeia
BP	British pharmacopeia
gm	Gram
Kg	Kilo gram
cm	Centimeter
ml	Milliliter
mEq/l	Mili equivalent per liter
gm/cm	Gram per centimeter

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OBJECTIVES OF PRESENT WORK

Drug is incompletely absorbed from GIT, thus its oral bioavailability is only about 50%. The incomplete absorption is mainly due to its lower lipid solubility. Literature shows that moderately polar drugs with low molecular weight can penetrate through the nasal mucosa to the greater extent than through gastrointestinal tract, thus the present work is aimed at studying the nasal permeation of Drug and improving its nasal permeation by the addition of penetration enhancer chitosan so that Drug could be successfully administered by nasal route.

However one shortcoming associated with the nasal route is high mucocilliary clearance and low retention of the drug for short period of time. Thus an attempt has been made to increase the retention time of the drug by incorporating chitosan, which shows temperature mediated phase transition in presences of sodium beta-glycerophosphate and possess mucoadhesive property as well.

Present investigation was planed with following objectives

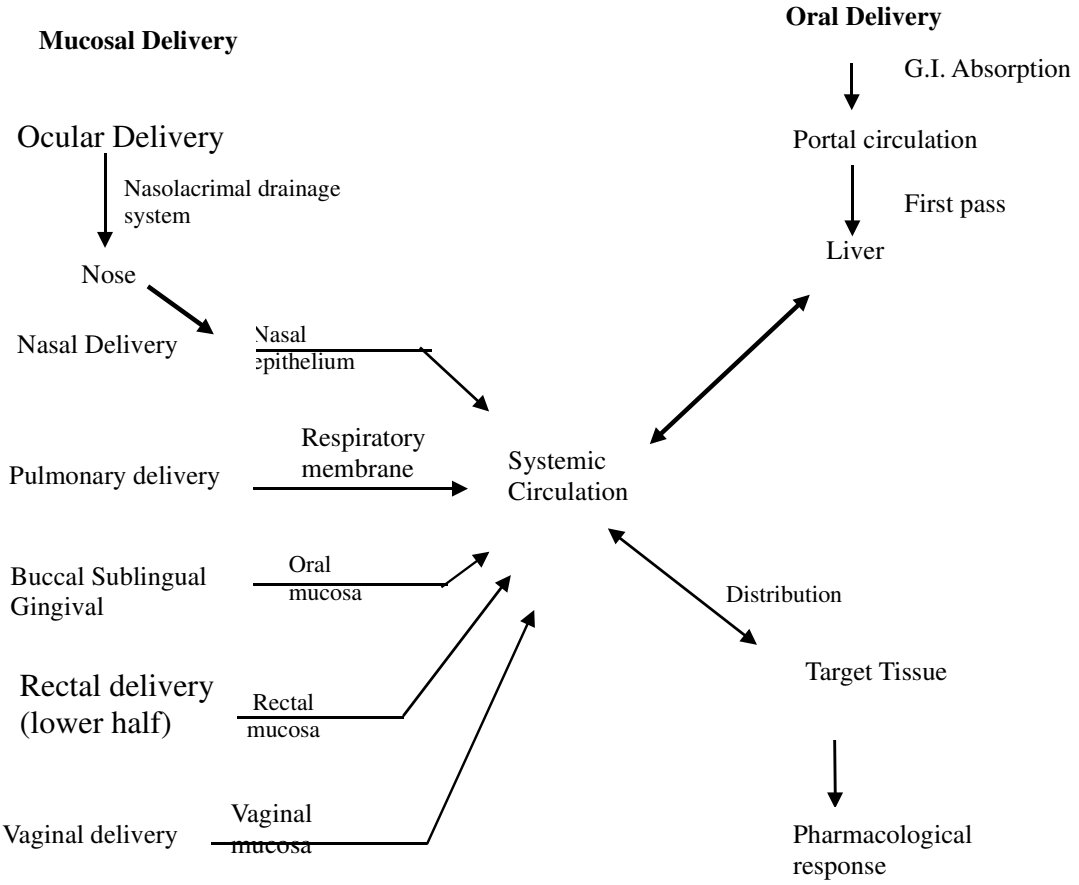
- To find the applicability of chitosan in the formulation of phase transition system.
- To study the effect of chitosan as nasal permeation enhancer.
- To study the mucoadhesive property of chitosan.

CH. I 1. INTRODUCTION [1,2,3,4,5,6]

Drugs have been administered nasally for therapeutic and recreational purposes since ancient times. Psychotrophic drugs and halucinogens were snuffed for their purposes

by the Indians of South America, and this practice is currently widespread among abusers of cocaine and heroine. Traditionally the nasal route has been used for delivery of drugs for local treatment of diseases such as nasal congestion, allergy and infections. The interest in and importance of the systemic effect of drugs administered via the nasal route have expanded over recent decades. Nasal administration offers an interesting alternative for achieving systemic drug effects to the parenteral route, which can be inconvenient, as parental route can be undesirable or impractical if a drug is intended for the treatment of chronic disease or oral administration, which can result in unacceptably low bioavailabilties because of significant degradation in the GIT due to enzymatic or acidic environment or metabolized to a high degree via the first pass effect in the liver. The non parenteral routes suitable for self administration of drug in an ambulatory settings includes nasal, buccal, pulmonary and transdermal. The nasal epithelium is a highly permeable monolayer, the submucosa is richly vascularised and hepatic first pass metabolism is avoided after nasal administration. Other attractive feature includes the rather large surface area (180 cm^2 because of the presence of large no. of microvilli) of the nasal cavity and the relatively high blood flow, which promotes rapid absorption, porous endothelial membrane and highly vascularised tissue providing an attractive site for rapid and efficient systemic absorption, furthermore, self medication is easy and convenient. Currently, nasal administration is used therapeutically for the systemic absorption of drugs in a variety of indications, including sumatriptan for migraine[7], the antidiuretic desmopressin for the treatment of diabetes insipidus[8] and oxytocin for the stimulation of breastmilk ejection. Other drugs still in the research and development pipeline, which have potential for administration nasally includes vitamin B12 or hydroxocobalamine, various benzodiazepines and the dopamine agonist apomorphine for patients with parkinsons

Figure 1: Various mucosal routes as potential pathway to by pass the hepato-gastrointestinal first pass elimination associated with oral administration.



1.1 Advantages of nasal drug delivery: [6,9,10,11]

Nasal Drug delivery provides a viable alternative for the administration of many pharmaceutical agents. Some of the major advantages offered by the nasal route include:

- Rapid absorption into the systemic circulation.
- Rapid onset of therapeutic action.
- Elimination of first pass hepatic metabolism.
- Avoids degradation of drugs in the gastrointestinal tract, resulting from acidic or enzymatic degradation.

- Rich vasculature and highly permeable structure of nasal mucosa results in higher bioavailability thus require lower doses of drug.
- More flexible dosing schedule and control of drug effects
- No pulmonary toxicity.
- Less drug degradation.
- Easily accessible, non-invasive route, thus better patient's compliance.
- Self-medication is possible through this route.
- Offer lower risk of overdose.
- Easy accessibility to blood capillaries.
- Direct transport into systemic circulation and CNS is possible.
- Does not have any complex formulation requirements.

Disadvantages of nasal drug delivery: [6,9,10,11]

- High molecular weight compounds cannot be delivered through this route (mass cut off ~1 K dalton).
- Large mucociliary clearance of the nasal mucosa may cause the poor absorption of certain drugs.
- Some therapeutic agents may be susceptible to partial degradation in the nasal mucosa or may cause irritation to the mucosa.
- Adversely affected by pathological conditions.
- Volume that can be delivered into the nasal cavity is restricted to 25-200 µl.
- Large interspecies variability is observed in this route.
- Limited understanding of mechanism and less developed model at this stage.

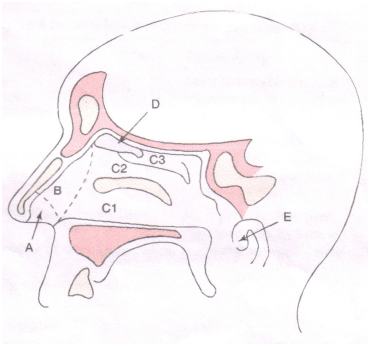
Overview of nasal mucosa [1,2,3,12,13,14]

Anatomy and function

Breathing and olfaction are the prime functions of the nasal cavity in humans and animals. Physiologically, the structure and function of this cavity are also related to the resonance of produced sounds, the filtration of particles, mucociliary clearance, immunological activities, and heating and humidification of the inspired air before it reaches the lungs.[1]

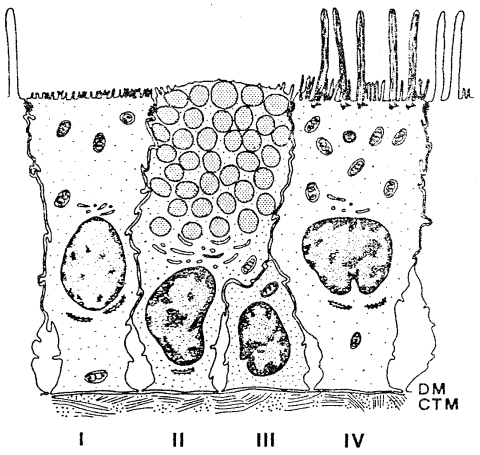
The nasal passage which runs from nasal vestibule (i.e. nasal valve) to the nasopharynx has a depth of approximately 12-14 cm. The nasal septum divides the nasal cavity into two unequal cavities. The septum consists mostly of cartilage and skin and therefore, the penetration of drug is low. The nasal cavity can anatomically be segregated into five different regions, nasal vestibule, atrium, respiratory area, olfactory region, and the nasopharynx as shown in Figure 2. The vestibular area serves as a baffle system, and its surface is covered by a common pseudo stratified epithelium where long hair may provide the function of filtering airborne particles. Secondly the olfactory region which is located on the roof of the nasal cavity in humans and forms about 10% of the total area of nasal cavity. Thirdly respiratory region consists of the inferior, middle and superior turbinate attached to the lateral wall, and is considered as major site of drug absorption into the systemic circulation. The respiratory area has a surface lined by a pseudo stratified columnar epithelium and is normally covered by a dense layer of mucus. These cells are covered by microvilli and the major part of these cells is also covered with cilia. Large number of microvilli results in increased surface area of 180 cm^2 responsible for relatively high absorptive capacity of the nasal cavity, whereas ciliated cells propel the mucus layer in a direction from the anterior towards the posterior part of the nasal cavity. Cilia beat with a frequency of 1000 strokes per minute and the mucus is transported at the rate of 5 mm per minute. The mucus layer is renewed every 15-20 min and hence the formulations applied to human nasal cavity are cleared with a clearance half-life of 15 minutes.

Figure 2 : Saggital section of the human nasal cavity, showing the nasal vestibule (A), Atrium (B), Respiratory Area: Inferior (C1), Middle (C2) and Superior (C3) turbinate, Olfactory region (D) and Nasopharynx (E).1.3.2 Nasal Epithelium [12]



The nasal cavity is highly vascularised and its membrane can be classified into two types: olfactory and non-olfactory. The olfactory epithelium is a pseudo stratified columnar structure. It consists of specialized olfactory cells, supporting cells, serous and mucosal glands. The nonolfactory part is a vascular membrane. Its surface is covered by ciliated pseudo stratified columnar epithelium. Numerous groups of microvilli can be seen microscopically among the group of cilia. All microvilli are of short club like appearances and there are approximately 500 microvilli on the surface of each ciliated cell. These cells with microvilli are called goblet cells. Another type of epithelial cells are observed in the free surface of the mucous membrane. They are rounded or elongated in shape and rough on the surface. These cells are defined as squamous cells.

Figure 3 : Trans membrane electron microscopic view of various cell types in the nasal epithelium.



Nasal Secretions [9,12]

The composition of the nasal secretions is complex and consists of a mixture of secretary materials from the goblet cells, nasal glands, and lacrimal glands and a transudate from plasma. In a clean, noninfected, nonallergic, and nonirritated nose, the mucosa is covered by a thin layer of clear mucus which is secreted from the mucous and serous glands in the nasal mucosa and sub mucosa. A total of approximately 1500-2000 ml of mucus is produced daily, which contains 90-95 % water, 1-2 % salt and 2-3 % mucin. The mucus has a two-layer composition: The watery (sol) layer is located immediately adjacent to the mucosal surface, and the mucous (gel) layer, which is more superficial. Normal Nasal secretions contain about 150 mEq/L of sodium, 40 mEq/L of potassium, and 8mEq/L of calcium as well as about 600 mg % of proteins, including 57 mg % of albumins and 133 and 50 mg % of immunoglobulin A (IgA) and G (IgG) respectively.

In addition to mucous glycoproteins, nasal secretions contain a variety of other proteins, lysozymes, enzymes, Ig A, Ig E, Ig G and albumins, kallikrein like substances, protease inhibitor, prostaglandins, as well as serum proteins like gamma A-globulin, gammaG -globulin, albumin and siderophilin.

The functions of mucus include:

- Acts as a retainer for the substances in the nasal duct
- Behaves as an adhesive
- Has water holding capacity
- Transports particulate matter
- Exhibit surface electricity activity
- Protect the mucosa
- Acts as mesh with permeability
- Allows heat transfer

Nasal mucociliary clearance [13,15]

Nasal mucociliary clearance is normal defense mechanism of the nasal cavity that clears mucus as well as substances adhering to the nasal mucosa (bacteria, allergens, and so on) and drains them into the nasopharyx for eventual discharge into the gastrointestinal tract. There are approximately five ciliated cells for each mucous cell, with an average of 200 cilia extending from every ciliated cell on the surface of pseudo stratified columnar epithelium. An individual cilium is approximately 5 µm in length and 0.2 µ in diameters, which moves at a frequency of about 20 beats/sec. nasal clearance proceeds at an average rate of about 5-6 mm/min. Normal mucociliary transit time in humans has been reported to be 12-15 min. Transit times more than 30 min are considered to be abnormal, and are indicative of impaired mucociliary clearance. Formulations administered to the human respiratory epithelium have been found to be cleared from the nasal cavity in ~ 21 min by mucociliary clearance.

Figure 4 : Mucocilliary clearance of the nose

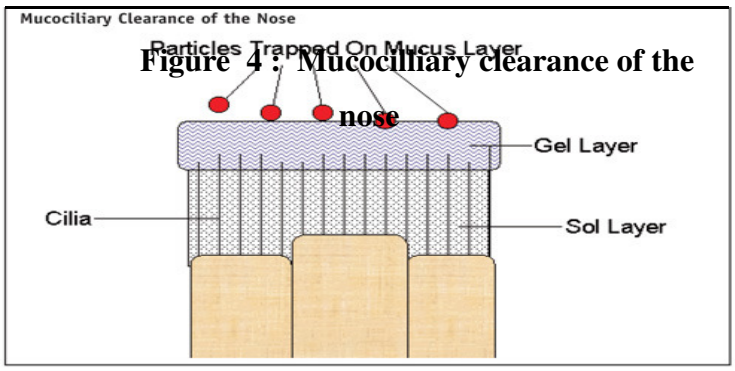


Figure 4 : Mucocilliary clearance of the nose

Nasal Enzymes [9,12,16]

Nasal mucus acts as enzymatic barriers to the delivery of drugs because of the presence of a large number of enzymes. They are cytochrome P-450 dependent monoxygenases, Lactate dehydrogenase, oxydoreductases, hydrolases acid phosphatase and esterase, NAD^+ dependent formaldehyde dehydrogenases and aldehyde dehydrogenase; leucine amino peptidase, phosphoglucomutase, glucose-6-phosphate dehydrogenase, aldolase, lactate dehydrogenase, isocitric dehydrogenase, glutamic transaminase and steroid hydroxylases. These enzymes are responsible for the degradation of drugs in the nasal mucosa and results in creation of a pseudo-first pass effect, which hampers the absorption of drugs. In spite of these hurdles, the nasal route is still considered to be superior to the oral route. The level of amino-peptidase present is much lower than that in the gastrointestinal tract. Various approaches have been used to overcome these degradations. These include the use of protease and peptidase inhibitors such as bacitracin, amastatin etc. Apart from using enzyme inhibitors, efforts are focused on designing prodrug to increase the stability and permeation of compounds

Although enzymes are known to exist in the nasal tissues, they do not appear to have a significant effect on the extent of absorption of most compounds except peptides. For example, the nasal bioavailability in animal and man of progesterone, testosterone, estradiol, naloxone, propranolol and butorphanol is almost 100 % of that

of the intravenous administration. The above drugs have an oral bioavailability ranging from 20-30 % for propranolol to 0 % for the other mentioned drugs. These low oral bioavailabilities are due to the extensive metabolism of the compounds in the gastrointestinal tract. The nasal administration of these compounds results in complete absorption because the level of the enzymes in the nasal tissue (mg/g) is very low and can be easily saturated with the drug.

Nasal pH [9,12]

The normal pH of the nasal secretions in the adult ranges approximately from 5.5 to 6.5, whereas in infants and young children it ranges from 5.0 to 6.7. During acute rhinitis, acute sinusitis, and in the more acute phases of allergic rhinitis, the pH of the nasal secretions was found to be on the alkaline side and then shifted back to acidity, when the stage of clinical resolution was reached. The cause of nasal pH can be altered by the influence of cold or heat. Cold air produces a drift towards alkalinity, whereas heat yields a drift toward acidity. Greater drug permeation is usually achieved at a nasal pH that is lower than the drugs pKa because under such conditions the penetrant molecules exist as unionized species. Because the pH of the nasal cavity can alter the pH of the formulation and vice-versa, the ideal pH of a formulation should be within 4.5-6.5.

Nasal blood flow[12,17]

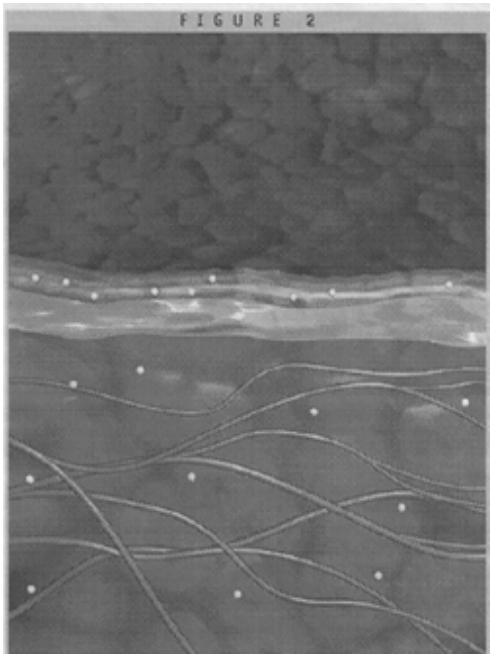
The nasal mucosa is highly vascular. The surface of epithelium is supplied with a dense network of erectile cavernous tissue which is particularly well developed over the turbinate and septum. The presence of venous sinusoids and arteriovenous anastomosis gives the nasal mucosa the distinction of being a highly permeable site.

The arterial blood supply to the nasal cavity is derived from both the external and internal carotid arteries. The terminal branch of the maxillary artery supplies the sphenopalatine artery which in turn supplies the lateral and medial wall of the nasal chamber. The anterior and posterior ethmoid branches come from the ophthalmic artery, which is a branch of the carotid artery. These vessels supply the anterior portion of the nose. Additionally, twigs from the facial artery supply the vestibule and anterior portion of the septum. Some vessels from the greater palatine artery pass

through the incisive canal of the palate to reach the anterior part of the nose. The veins of the nasal cavity drain into the sphenopalatine foramen and then into the pterygoid plexus. Some other veins accompany the ethmoid arteries and join the superior ophthalmic vein. Veins which are anterior in the nose drain into the facial vein. The richly supplied vascular nature of the nasal mucosa, coupled with its low barrier to drug permeation, makes the nasal route of administration attractive for a number of drugs, both peptide and nonpeptide drugs. In addition, the olfactory region provides a potential advantage whereby a drug may be exposed to neurons that may facilate its access into the cerebral spinal fluid, when administered nasally.

Constriction of the blood vessels would decrease blood flow and blood content in the nasal mucosa, whereas vasodilation would yield the opposite response. The penetration of the drug through the sinus mucosa is partly influenced by the blood flow in the region under normal and pathological conditions

Figure 5 : Diagram showing highly vascularised nasal cavity



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Nasal Pathophysiology [12]

Diseases such as the common cold, rhinitis, atropic rhinitis, nasal polyposis are usually associated with mucociliary dysfunction, hypo and hyper secretions, and irritation of the nasal mucosa, which can influence drug permeation and subsequently, the therapeutic efficacy of the drugs administered nasally. In some subjects with a severe nasal allergy, an excessive response of the secretary system to irritants could wash away the drug solution administered into the nasal cavity before the drug is absorbed by the nasal membrane.

Tight Junction (TJ) [10,18]

TJs are structure that form a barrier between adjacent epithelial cells with a narrow band just beneath the apical surface, and are found in all tissues of the body. They perform two vital function: as a barrier or gate to the movement of molecule between cells in the paracellular space, and as a fence to prevent diffusion of integral membrane proteins between the apical and basolateral surfaces, thus preserving, for example, the special function of receptor mediated endocytosis at the apical surface and exocytosis at the basolateral surface. High molecular weight drugs need to pass through TJ barriers in order to become systemically available and to move to their site of action as part of the bodies normal activity, TJs selectively modulate paracellular permeability by opening and closing in response to various signals inside and outside of cells, including responding to cytokines, immune cells, nutrients, calcium depletion and lipid modifying agents. TJs consist of a variety of integral membrane and peripheral, or associated, proteins, which are anchored in the membranes of two adjacent cells and interact across the paracellular space by noncovalent forces .In the cytoplasm, TJ membrane proteins interact with scaffold proteins to connect them with the cellular cytoskeleton and various signal transduction and transcriptional pathways involved in the regulation of TJ function. Dysregulation of TJ function occurs in a variety of diseases, particularly inflammation, cancer and CNS pathologies where normal tissue permeability and cell adhesion interactions are altered.

Transmembrane Proteins of the Junctional Complex

Epithelial cells characteristics of the nasal mucosa, like other tissues, are joined together by TJs. The closely associated adherens junction is found on the basolateral side but is not circumferentially continuous, like the TJ, and therefore does not contribute significantly to the epithelial barrier properties. The cell membranes of adjacent cells are intimately connected by proteins of the junctional complexes to an extent that one can measure a significant transepithelial electrical resistance (TEER). Freeze fracture electron microscopy (but not thin sections) visualises TJs as a network of strands that appear as rows of 10 nm particles within the plane of the plasma membranes of the neighbouring cells. These strands have been predicted to contain pores that dynamically open and close. Three major types of integral proteins comprise TJs: Occludin, claudins and junction adhesion molecules (JAM) These junctional proteins are involved in cell-cell adhesion and are dynamically regulated .A group of scaffolding proteins, including a family of zonula occludens (ZO) proteins, connect TJs and AJs to the cytoskeleton and mediate intracellular signaling. A number of additional cytosolic and nuclear proteins interact directly or indirectly with TJ scaffolding proteins and are also involved in regulating diverse functions including paracellular permeability cell polarity etc claudins may be the single most important component of the TJ because they alone can form TJ strands. Claudins play a key role in regulating ion flux as major components of paracellular channels,where indivisual claudins function as ion–specific pores. E-cadherin is a 120-k Da a transmembrane protein, which is a constituent of the AJ. It is important for initiating and maintaining cell-cell contacts and is required for the formation of and maintenance of TJs.

Chitosan has been shown to disrupt intercellular TJs, thus increasing permeability by translocation of TJ proteins from the membrane to the cytoskeleton. Immuno fluorescent localization of ZO-1 revealed loss of membrane associated ZO-1 and occludin from the cytosolic and membrane fractions into the cytoskeletal fraction.

Drug Absorption Through the Nasal Mucosa [13,20]

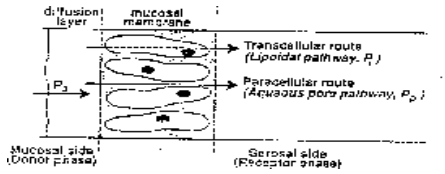
The first in the absorption of drugs from the nasal cavity is passage through the mucus. Small uncharged particles easily pass through this layer. However larger particles may find it more difficult to cross. Mucin the principle protein in the mucus has the potential to bind solutes, hindering diffusion. Additionally structural changes in the mucus layer are possible as a result of environmental changes, (i.e. pH and temperature). After a drug passage through the mucus, there are several mechanisms for absorption through the mucosa.

Mechanism of drug absorption:

As seen with the other epithelium in the body, absorption across nasal epithelium can occur by one or combination of mechanisms. Following two mechanisms have been considered predominantly. The first mechanism involves an aqueous route of transport, which is also known as the paracellular route. Drugs are believed to pass through the epithelium via the gaps or pores between the cells (the tight junction). This route is slow and passive and this pathway is especially suited for smaller hydrophilic molecules. Although, the tight junctions are dynamic structures that can open and close to certain extent, the size of these channels is less than 10 A^{o}. There is an inverse log correlation between intranasal absorption and the molecular weight of water-soluble compounds. Hence, the paracellular route will be less efficient for large molecules and is dependent upon the molecular weight of the drug with a general molecular size cut – off of 1000 Dalton.

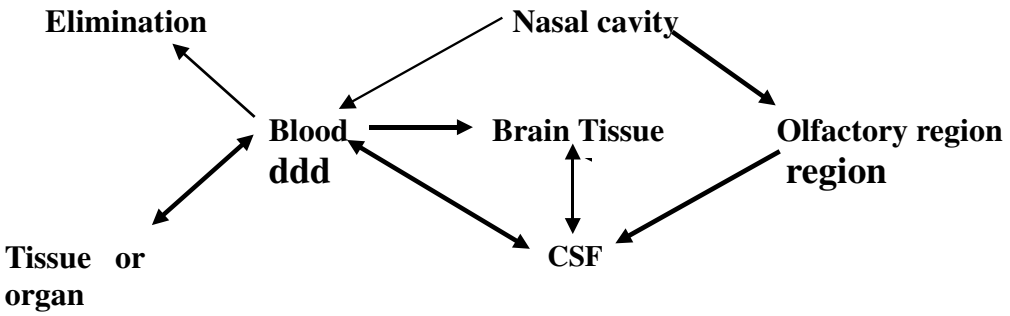
The second mechanism involves transport through a lipoidal route that is also known as the transcellular process and is responsible for the transport of lipophilic drugs by an efficient concentration dependent passive diffusion process, by receptor or carrier mediation and by vesicular transport mechanism. This pathway is especially suited for small lipophilic molecules or large molecules.

Figure 6 : Diagram showing the physical model for transmembrane permeation across a mucosal membrane



Intracellular axonal transport of drugs through olfactory neuron cells is also one of the mechanisms, responsible for transport of drugs primarily to the olfactory bulb. The olfactory epithelium is known to be a portal of entry for substances into the central nervous system (CNS) and peripheral circulation. The transport of drugs across the nasal membrane and into the blood stream may involved either passive diffusion of the drug molecules through the pores in the nasal mucosa or some form of non-passive transport. The can enter into the CNS when administered in the nasal cavity, a proportion is transported through the olfactory lobes of the brain, or in some cases, further into the parenchyma of the brain. It has been show that the rate and degree of transport and deposition are highly dependent on the physicochemical properties of the drugs, especially molecular weight and the lipophilicity. Drug transport routes from nasal cavity are depicted in Figure 7. When drugs are administered nasally the drug will normally be rapidly cleared by the mucocilliary mechanism. Some of the drug will be absorbed into the blood stream from where it reaches the systemic circulation directly and subsequently is eliminated from the blood by crossing the blood brain barrier, but can also be eliminated from CSF into the brain.

Figure7:possible route of transport of drug administered nasally



1.8.2 Factors influencing the absorption of drugs through nasal epithelium[9,17,21]

Figure 7 : **Possible route of transport of drug administered nasally**

Factors influencing absorption are related to nasal physiology, physicochemical characteristics of drugs and formulation aspects.

I. Biological:

A) Structural features

B) Biochemical changes

C) Physiological factors

- Blood flow
- Nasal secretions
- pH of the nasal cavity
- Mucocilliary clearance and ciliary beat frequency

D) Pathological conditions

E) Environmental factors

- Temperature
- Humidity

II. Physicochemical properties of drugs

- Molecular weight
- Size
- Solubility
- Lipophilicity
- Pka and partion coefficient

III. Physicochemical properties of formulation

- Dosage form
- Viscosity
- PH and mucosal irritancy
- Osmolarity
- Volume of solution applied

IV. Device related

- Particle size of the droplet/powder
- Size and pattern of disposion

Physiological factors include firstly mucociliary clearance is one of the major factor responsible for the clearance of the drugs from the nasal cavity, it involves combined action of mucus layer and cilia, tips of cilia are in contact with and transport the superficial viscoelastic mucus layer towards nasopharynx while less viscous lower layer of mucus is relatively stationary. Secondly broad ranges of metabolic enzymes are present in the nasal mucosa. This can limit bioavailability of nasally administered drugs; however level of activity of these enzymes is lower as compared to that found in GIT and liver. Moreover pathological conditions like rhinitis, common cold can also affect absorption of drugs from nasal cavity. pH of nasal cavity also affects permeation of drug. A change in the pH of mucus can affect the ionization and increase or decrease the permeation of drug, depending on the nature of the drug.

Physicochemical characteristics of drugs:

Various physicochemical characteristics of drug can also affect nasal absorption of the drug.

Molecular Weight and Size: [13,17,20]

Extent of the absorption of the drug depends on molecular weight particularly for hydrophilic compounds. Nasal route is suitable for efficient delivery of drugs up to 1000 Daltons. Absorption reduces the significantly if the molecular weight is greater than 1000 Daltons except with the use of penetration enhancers. It has been reported that a good linear correlation exists between the log percentage drug absorbed nasally and the log molecular weight of water soluble compounds suggestion the participation of aqueous channels in the nasal absorption of water soluble molecules.

It has been reported that particle size greater than 10 µm are deposited in the nasal cavity. Particles that are 2 to 10 µm can be retained in the lungs, and particles of less than 1 µm are exhaled.

Solubility and Dissolution:

Drug solubility is a major factor in determining absorption of drug through biological membranes. It not only limits the drug absorption per se, it can also limit a formulator's ability to formulate a product if the drug is not sufficiently soluble in the desired vehicles. As nasal secretions are more watery in nature, a drug should have appropriate aqueous solubility for increased dissolution. Particles deposited in the nostrils need to be dissolved prior to absorption. If the drug remains as particles in nostrils, or if they are cleared away from the nasal cavity, one may not observe absorption of the drug.

Chemical form:

The chemical form in which a drug is presented at the nasal mucosa can be important in determining its absorption. For example, conversion of a drug into a salt or ester form can alter its absorption. This phenomenon is associated with the increase in

lipophilicity following esterification, which increased the rate and extent of nasal absorption.

Partition coefficient and pKa:

Jiang *et al.* (1997) conducted a study to find out the quantitative relationship between the physiochemical properties of drugs and their nasal absorption, using diltiazem hydrochloride and paracetamol as model drugs. The result showed that a quantitative relationship existed between the partition coefficient and nasal absorption constant. As per the pH partition theory, unionized species are absorbed better compared with ionized species and same holds true in the case of nasal absorption. The extent of absorption is PH dependent, being higher at a pH lower than the pKa and decreases beyond the pKa.

A relationship between the lipophilicity and absorption rate constant of mucosal absorption of progesterone was shown by Corbo *et. al*. In general, the authors found that the nasal absorption increase with the lipophilicity of the permeant. Various studies indicate that the drug concentrations in the cerebrospinal fluid (CSF) rise with an increase in lipophilicity or partition coefficient of the drugs. The nasal absorption of weak electrolytes such as salicylic acid and aminopyrine was found to be highly dependent on their degree of ionization. Although for aminopyrine, the absorption rate increased with the increase in pH and was found to fit well to the theoretical profile, substantial deviations were observed with salicylic acid. The authors concluded that perhaps a different transport pathway, along with the lipoidal pathway, existed for salicylic acid. Similarly when the absorption of benzoic acid was studied at PH 7.19 (99.9 % of the drug existed in ionized form) it was found that >10 % of drug was absorbed indicating that the ionized species also permeates through nasal mucosa. Based on all of these observations, the authors discounted partition coefficients as a major factor governing nasal absorption and supported that other transport pathways for hydrophilic drugs might be of importance.

Factors Related to Formulation [9,17]

Drug concentration, dose and dose volume

Drug concentration, dose and dose volume of administration are three interrelated parameters that impact the performance of the nasal delivery system. Nasal absorption of L-Tyrosine was shown to increase with drug concentration in nasal perfusion experiments. However, in another study, aminopyrine was found to absorb at a constant rate as a function of concentration.

Several studies have reported the effect of drug 'dose' on nasal absorption, e.g. calcitonin, GnRH agonist, desmopressin, secretin. In general, higher nasal absorption or therapeutic effect was observed with increasing dose. It is important to note how the dose is varied. If the drug is increasing by increasing formulation volume, there may be a limit as to what extent nasal absorption can be increased. The nostrils can retain only a limited volume, beyond which a formulation will drain out of the nasal cavity. The ideal dose volume range is 0.05-0.15 ml with an upper limit of 0.20 ml.

Physical form of formulation:

Nasal drug absorption depends on the physical form of the formulation. The important parameter in formulation development is viscosity of the formulation. Generally a more viscous formulation will provide less efficient systemic nasal drug delivery. Harris et al. studied the nasal delivery of desmopressin and reported that although the addition of the viscous agents to nasal formulations may produced a somewhat more sustained effect. It would seem logical that more viscous formulations e.g. gels should be more appropriate for locally acting drugs. One may also consider developing gel type formulations for those drugs, which cause unpleasant taste in the mouth via a nasal drip of solution or spray formulations. Nasal drip would be minimized from viscous formulations.

Formulation pH:

The pH of the formulation as well as that of nasal surface, can affect a drug's permeation. The pH of the nasal formulation is important for the following reasons:

- To avoid irritation of the nasal mucosa.
- To allow the drug to be available in unionized form for absorption.
- To prevent the growth of pathogenic bacteria in the nasal passage.
- To maintain functionality of excipients such as preservatives and
- To sustain normal physiological ciliary movement.

Lysozymes are found in nasal secretions, which is responsible for destroying certain bacteria at acidic pH. Under alkaline conditions, lysozyme is inactivated and the nasal tissue is susceptible to microbial infection. It is therefore advisable to keep the formulation at a pH of 4.5 to 6.5 keeping in mind the physicochemical properties of the drug as drugs are absorbed in the unionized form and also to avoid nasal irritation.

Buffer capacity:

Nasal formulations are generally administered in small volumes ranging from 25 to 200 µl with 100 µl being the most common dose volume. Hence, nasal secretions may alter the pH of the administered dose. This can affect the concentration of un-ionized drug available for absorption. Therefore, an adequate formulation buffer capacity may be required to maintain the pH *in-situ.*

Osmolarity:

Drug absorption can be affected by tonicity of the formulation. Shrinkage of the epithelial cells has been observed in the presence of hypertonic solutions. Hypertonic saline solutions also inhibit or cease ciliary activity. Low pH has a similar effect as that of hypertonic solutions. Generally an isotonic formulation is preferred.

Gelling / viscofying agents or gel forming carriers:

Some formulations need to be gelled or made more viscous to increase nasal residence time. According to a study by Pennington *et al.* increasing the solution

viscosity may provide a means of prolonging the therapeutic effect of nasal preparations. Suzuki et al. showed that a drug carrier such as hydroxypropyl cellulose was effective for improving the absorption of low molecular weight drugs but did not produce the same effect for high molecular weight peptides. Use of a combination of carriers is often recommended from a safety (nasal irritancy) point of view.

Solubilizers:

Aqueous solubility of a drug is always a limitation for nasal drug delivery in solution. Conventional solvents or co-solvents such as glycols, small quantities of alcohol, Transcutol, (diethylene glycol monoethyl ether), medium chain glycerides and Labrasol (saturated polyglycolyzed C_8-C_{10} glycerides) can be used to enhance the solubility of drugs. Other options include the use of surfactants or cyclodextrins such as HP-β-Cyclodextrins that serve as a biocompatible solubilizer and stabilizer in combination with lipophilic absorption enhancers. In such cases, their impact on nasal irritancy should be considered.

Preservatives:

Most nasal formulations are aqueous based and need preservatives to prevent microbial growth. Parabens, benzalkonium chloride, phenyl ethyl alcohol, EDTA and benzoyl alcohol are some of the commonly used preservatives in nasal formulations. Preservatives are based in small quantities and are not likely to affect drug absorption.

Antioxidants:

Depending upon the stability profile of a given drug in the formulation chosen, it may be necessary to use antioxidants to prevent drug degradation. Commonly used antioxidants are sodium metabisulfite, sodium bisulfite, butylated hydroxytoluene and to copherol. Usually, antioxidants are used in small quantities and they may not affect drug absorption or cause any nasal irritation. Chemical/physical interactions of antioxidants and preservatives with drugs, excipients, manufacturing equipment and packaging components should be considered as a part of formulation development program.

Humectants:

Many allergic and chronic diseases are often connected with crusts and drying of mucous membranes. Certain preservatives/antioxidants among the other excipients are also likely to cause nasal irritation especially when used in higher quantities. Adequate intranasal moisture is essential for preventing dehydration. Therefore, humectants can be added especially in gel-based nasal products. Humectants avoid nasal irritation and are not likely to affect drug absorption. Some common humectants used include glycerin, sorbitol and mannitol.

Absorption Enhancers [1,22]

When it becomes difficult for a nasal product to achieve its required absorption profile, the use of absorption enhancers is recommended. The selection of absorption enhancers is based upon their acceptability by regulatory agencies and their impact on the physiological functioning of the nose. Absorption enhancers may be required when a drug exhibits poor membrane permeability, large molecular size, lack of lipophilicity and enzymatic degradation by aminopeptidases. Once a suitable enhancer is identified, its optimal concentration should be experimentally determined. Generally higher concentrations of enhancers are likely to result in nasal irritation and damage to the nasal mucosa. On the other hand, lower enhancer concentrations would generally provide lower or no improvement of absorption. The various compounds investigated as enhancers in nasal drug delivery research are mentioned in Table 1.

Table 1 : Various compounds investigated as enhancers in nasal drug delivery research

Surfactants	Sodium dodecyl sulfate (SDS) Polyoxyethylene-9-lauryl ether Phosphatidylcholines
Complexing and Chelating agents	Ethylene diamine tetraacetic acid(EDTA)
Cyclodextrins and derivatives	α-, β-, γ-cyclodextrin DMβ-, HPβ-cyclodextrin
Bile saltsSodium Tauradihydrofusidate (STDHF) Fusidic acid derivatives	Sodium taurocholate Sodium glycocholate
Dry microspheres	Degradable starch microsphere Dextran microspheres

Ideal Characteristics of Absorption Enhancers

The following characteristics should be considered in choosing absorption enhancers:

- The enhancer should be pharmacologically inert at concentrations used.
- It should be nonirritating, nontoxic and nonallergic.
- If the enhancer has any effect on the nasal mucosa, it should be completely reversible.
- The enhancer should be a potent absorption promoter therefore requiring only small amounts to be used.
- It should be compatible with the drug and formulation adjuvants
- It should be able to remain in contact with the nasal mucosa long enough to achieve a maximal effect
- The enhancer should not have any offensive odor or taste
- It should be relatively inexpensive and readily available.

Mechanism of Nasal Drug Absorption Enhancers:

The enhancers evaluated to date appear to act by a wide range of mechanisms, including perturbation of lipid membranes, facilitation of leakage of lipids and proteins from the membranes, tight junction regulation, and chelation of Ca2+ ions in the cell membranes.

Precise mechanisms of enhancer effect are not known. However, it is generally believed that enhancers may show their actions via one or both of the following mechanisms:

Physicochemical effects:

Some enhancers can alter the physicochemical properties of a drug in the formulation. This can happen by altering the drug solubility, drug partition coefficient, or by weak ionic interactions with the drug. This mechanism of drug absorption enhancement is desirable because it can be effective with the lowest potential of toxicity.

Membrane effects:

Many enhancers show their effects by affecting the nasal mucosal surface. It should be emphasized that these effects are not necessarily harmful. In most cases the enhancer's effects are transient with no lasting or pathological consequences. The final decision should take into consideration the benefit to risk ratio.

Generally, the absorption enhancers act via one of the following mechanism:

- Inhibit enzyme activity;
- Reduce mucus viscosity or elasticity;
- Decrease mucociliary clearance;
- Open tight junctions; and
- Solubilize or stabilize the drug.

Nasal Formulations [6,23,24,25,26,27,28,29,30]

Several new preparations mentioned below have been developed for nasal route not only to prolong the contact time of the vehicle on the nasal mucosal surface but also to slow down the drug clearance unlike nasal drops

- Suspension[9]
- Powders[32]
- Emulsions and ointments[33]
- Microsphere[28,34,35]
- Liposomse and Proliposomes[36,37]
- Mucoadhesive drug delivery system[38,39,40]

Mucoadhesive Drug Delivery System [38,39,40]

A common way of increasing residence time is by adding bioadhesive polymers to the nasal solutions in order to increase the viscosity and thereby decrease the rate of drainage. Mucoadhesive drug delivery systems that utilized the property of bioadhesion of certain polymers, which becomes adhesive on hydration and hence can used for targeting a drug to a particular region of the body for extended periods of time.

In general it is assumed that the polymers and microspheres interact with mucus and the tissue surface with a resulting increase in contact time. Many commonly used bioadhesive polymers are anionic or nonionic compounds with active hydrophilic functional groups, which can form hydrogen bond along the polymer chain and with mucus.

Bioadhesion and Mucoadhesion:

The idea of using bioadhesive polymers to prolong the contact time in the mucosal routes of drug delivery was introduced in the early 1980s and, since then, it has attracted considerable attention from pharmaceutical scientists. The potential of the drug delivery system to localize a drug at the site of absorption for an extended period of time and to promote intimate contact between the formulation and the underlying absorbing tissue has great appeal to both local and systemic effects. Good considered Bioadhesion to be the phenomenon in which two materials, at least one being of biological nature, are held together for extended periods of time by interfacial forces. Bioadhesion has been defined by Longer and Robinson as the attachment of synthetic or biological macromolecules to a biological tissue. If the adhesive attachment is to a mucous coat, the phenomenon is referred to as Mucoadhesion.

Mechanism of Mucoadhesion

For Bioadhesion to occur, a succession of phenomena, whose role depends on the nature of the bioadhesive is required. Following few steps are involved in Mucoadhesion process.

1. Spreading, wetting and swelling of the dosage form at the mucous surface, initiates intimate contact between the polymer and the mucous layer.
2. Interdiffusion and interpenetration take place between the chains of the mucoadhesive polymer and the mucous gel network, creating a greater area of contact.
3. Entanglement and secondary chemical bonds are formed between the polymer chains and mucin molecules.

It can be noted that, for polymer gels that are already in equilibrium swelling, the wetting and swelling step is unlikely to be involved.

The component of the mucus involved in interactions is the mucin molecules. These are glycoproteins of high molecular weight (in the range of $1\text{-}50.10^6$ Dalton) present in the concentration of 0.5-5 %, which are also responsible for the viscoelastic properties of the mucus. The mucin is negatively charged at physiological PH because

of sialic acid residues in the oligosaccharide units. On a molecular level, mucoadhesion can be explained based on molecular interactions. Hydrogen bonds are often considered to be the most important of the types of the secondary chemical bonds that can be formed in the mucoadhesion process. Other types of bonds that might be involved include ionic bonds and Vander Waals interactions.

Factors affecting Mucoadhesion

The mucoadhesive power of a polymer is affected by the nature of the polymer and also by the nature of the surrounding media. The factors influencing the mucoadhesion are as follows,

I. Polymer related factors

- Molecular weight
- Concentration of active polymer
- Flexibility of polymer chains
- Special confirmation
- Swelling

II. Environment related factors

- pH of the polymer-substrate interface
- Applied strength
- Initial contact time

III. Physiological factors

- Mucin turnover
- Disease state

Methods for measuring mucoadhesion

Several test methods have been reported in literature for studying mucoadhesion. These tests are important during the design and development of a bioadhesive controlled release system as they ensure compatibility, physical and mechanical stability, surface analysis and bioadhesive bond strength. The methods reported are as follows.

I*) In- vitro/Ex vivo* methods

- Methods based on measurement of tensile strength.
- Methods based on measurement of strain strength.

II) Other *in- vitro* methods:

- Adhesion weight method
- Fluorescent probe method
- Flow channel method
- Mechanical spectroscopic method
- Falling liquid film method
- Colloidal gold staining method
- Viscometric method
- Thumb test
- Adhesion number
- Electrical conductance

III) *In- vivo* methods:

- Use of radio isotope
- Use of gamma scintigraphy.

1.11.5 Advantages of mucoadhesive drug delivery systems:

Mucoadhesive dosage forms have distinct advantages when compared to conventional dosage forms.

1. These dosage forms are readily localized in the region applied to improve and enhance the bioavailability of drugs.
2. The dosage forms facilitate intimate contact of the formulation with the underlying absorption surface. This allows modification of the tissue permeability for absorption of macromolecules such as peptides and proteins. Inclusion of penetration enhancers such as sodium glycholate, sodium taurocholate and protease inhibitors in the mucoadhesive dosage forms resulted in the better absorption of the peptides and proteins.
3. Mucoadhesive dosage forms also prolong residence time of the dosage form at the site of application and absorption to permit once or twice a day dosing.

Mucoadhesive polymers

Bioadhesive polymers have been used extensively in nasal drug delivery systems to provide dosage forms retention. Bioadhesive polymers are defined as polymers that can adhere to a biological substrate. The term mucoadhesion is applied when the substrate is mucosal tissue. Diverse classes of polymers have been investigated for their potential use as mucoadhesive.

Mucoadhesive polymers are water soluble and water insoluble polymers which are swellable networks joint by cross linking agents. The polymers should possess optimal polarity to make sure it is sufficiently wetted by the mucus and optimal fluidity that permits the mutual adsorption and interpenetration of polymer and mucus to take place. Ideal polymers for mucoadhesive drug delivery system should have the following characteristics;

- The polymers and its degradation products should be nontoxic and nonabsorbable from the gastrointestinal tract.
- It should be a nonirritant to the mucous membranes.
- It should preferably form a strong noncovalent bond with the mucin epithelial cell surface.
- It should adhere quickly to moist tissue and should possess some site specificity.
- It should allow easy incorporation of the drug and offer no hinderance to its release.
- The polymer must not decompose on storage or during shelf life of the dosage form.
- The cost of the polymer should be not to high, so that prepared dosage form remains competitive.

Table 2 : Mucoadhesive polymers used in Nasal drug Delivery

Polymers	Bioadhesive property
Carboxy methyl cellulose	+ + +
Carbopol 934	+ + +
Polycarbophil	+ + +
Tragacanth	+ + +
Poly(acrylic acid/divinyl benzene)	+ + +
Sodium alginate	+ + +
Hydroxy ethyl cellulose	+ + +
Gum karaya	++
Thermally modified starch	++
Pectin	++
Polyvinyl pyrrolidene	+
Acacia	+
Polyethylene glycol	+
Psyllium	+
Amberlite-200 resin	+
Hydroxypropyl cellulose	+
Chitosan	+

The interaction between the mucus and bioadhesive polymers is a result of physical entanglement and secondary bonding mainly hydrogen bonding and vander walls attraction. These forces are related to the chemical structure of the polymers. The functional groups available on the surface of the polymer conformation favoring bioadhesion include hydroxyl, carboxyl, amines and amides. The bioadhesive polymers must have a critical molecular weight and an adequate length to allow chain interpenetration. Anionic polymers are usually preferred due to negatively charged mucin at physiological pH.

Phase Transition System [41,42]

There are polymeric solutions prepared from water soluble polymers that are able to form gels after application to delivery sites that is they gels once in position.

Preferred excipients for liquid compositions is one that allows the composition to be administer as a mobile liquid and in the nasal cavity will cause the liquid composition to gel, thereby providing a bioadhesive effect which acts to hold the drug at the absorptive surface for an extended period of time. A vitamin *B12* gel has been recently developed as a prescription product.

To solve the problems of conventional nasal drops, it would be desirable to developed a phase transition system which,

- Forms a gel at physiological condition
- Has suitable gel strength, not to leak out from the nasal cavity after nasal administration.
- Has a suitable bioadhesive force so as to increase the residence time.

Advantages of phase transition system:

The main advantage of using phase transition system is that,

- They are fluid like prior to contact with the mucosa and thus can be readily administered as a drop or by a spray device.
- The formulation adopts semisolid properties when gelling is induced by some physiological stimulus at the site of administration, which increases residence time due to mucoadhesive property.
- Bioavailability increase due to increase in residence time.
- Reduced the dosing frequency

These systems consist of polymers that exhibit sol to gel phase transitions due to a change in specific physiological parameter (pH, temperature) in environment of the nasal cavity. Depending on the method employed to cause sol to gel phase transition in nasal cavity, the following three types of systems are recognized.

- pH triggered systems- Cellulose acetate hydrogen phthalate, carbopol, chitosan.
- Ion activated systems- Gelrite, alginates.
- Temperature dependent systems- Pluronic and tetronics

Such systems have gain significant interest among the formulators within the pharmaceutical field as drug delivery vehicle and have been evaluated for several administration routes as nasal, ophthalmic, oral, dermal, rectal, vaginal, and parenteral.

CH. II. LITERATURE REVIEW.

- Illum, *et al*., studied intranasal delivery of morphine, the article describes the development of novel nasal morphine formulations based on chitosan showing 5 to 6 fold increase in bioavailability over simple morphine solution.[19]
- Aikawa, K., *et al.,* developed nasal formulations of polyvinylacetal diethylaminoacetate (AEA) and the effect of AEA concentration on drug release was evaluated in *in-vitro* and *in vivo* experiments. Hydrogel formation on mucous membrane was also visually confirmed in rat nasal cavity. Such gels would swell significantly when in contact with the mucosa (the pH of the mucosa is almost 7.0) and the release of the drug in a continuous fashion while adhering to the mucosa. From the results they concluded that AEA preparations which facilitate instillation into the nose but which form hydrogel on the mucous membrane are potentially useful for controlled release nasal delivery system.[25]
- Mao, S., *et al.,* studied melatonin starch microspheres for intranasal administration, which could be capable of providing a fast onset and controlled release behavior. The deposion and subsequent clearance of the drug from the nose was investigated by gamma scintigraphy and absolute bioavailability was calculated. *In vitro* release experiments showed that melatonin was released from the microspheres in a sustained manner. $T_{1/2}$ of the pure melatonin was 5.6 min., whereas a value of 12.3 min. was observed for melatonin starch microspheres. Nasal clearance studies showed that > 80% of the radioactivity from the starch microspheres was present in the nasal mucosa 2 hours after administration compared to only 30 % radioactivity from the solution. The absolute bioavailability of the melatonin starch microspheres after intranasal administration was 84.07 %.[29]
- Nagai, T., *et al.,* studied powder dosage of insulin for nasal administration. The results showed that addition of bioadhesive polymers in a powder nasal dosage form of insulin resulted in an increase in bioavailability as compared to an insulin solution.[31]

- Illum *et al.* in their *in vivo* study using formulations using bioadhesive microspheres for nasal administration reported that, the intranasal clearances of three microsphere systems, albumin, starch, and DEAE-dextran microspheres were much slower than the intranasal clearances of the control solution and powders. Half time for the clearance of the microsphere systems were 3 hours or longer while the half times for the clearance of the control solution or powders were approximately 15 minutes.[35]

- Chenite, A. *et al.,* studied novel injectable neutral solutions of chitosan. This study reported for the first time the use of polymer/polyol salt aqueous solution as gelling systems, suggesting the discovery of a prototype for a new family of thermally gelling, highly compatible with biological components.[43]

- Shaoyun**,** Yu. *et al*., studied nasal insulin delivery in the chitosan solution. The investigation described the effect of chitosan concentration and absorption enhancers in the chitosan solutions on nasal insulin delivery showing a significant effect on the insulin nasal delivery. This study showed that the combination of chitosan and HP – β cyclodextrin is most effective in enhancing the absorption of insulin in nasal delivery system compared to the formulation containing 1 % chitosan alone or 5 % HP – β cyclodextrin alone.[44]

- Chenite, A. *et al.,* studied rheological characterization of thermogelling chitosan / glycerophosphate solution.The paper discusses about the temperature triggered hydrogel formation by chitosan coupled with sodium beta glycerophosphate. [45]

- Hussain, *et al.,* studied nasal bioavailability of propranolol and its salt from different formulations in rats, dogs, and human and concluded that in all three species, nasal absorption was as effective as intravenous injection and much superior to oral absorption. [46]

- Hussain, A. *et al.,* in their study of nasal absorption of propranolol from different dosage forms in rats and dogs demonstrated the utility of methylcellulose with the report that the addition of 3 % methylcellulose to a

nasal solution of propranolol produced a sustained blood level over an extended time period. [47]

- Anne, P., *et al.,* formulated a injectable poloxamer based gel of lidocaine hydrochloride and ibuprofen Na and studied the effect of drug release from the poloxamer based gel and poloxamer gel with different additives like HPMC, sodium carboxymethylcellulose, and dextran. The study was carried out *in vitro* using porcine dura mater membrane. The result showed that cellulose additives significantly prolonged ibuprofen release, whereas other additives were found to have slight release increasing effect on lidocaine as compared with poloxamer gel. From the result it was concluded that the structural differences of the gel, more than macroviscosity seems to regulate the release of drugs. The compact gel depot acted as the rate limiting step, and significantly prolonged the dural effects of the gels demonstrated the possibility for interactions between dural membrane and the gel.[48]

- Paavola, A., *et al.,* studied controlled release injectable liposomal gel of ibuprofen for epidural analgesia and investigated the possibility of using liposomal systems to control the release and dural permeation of ibuprofen. The liposomal gel consisted of poloxamer 407 and the liposomal solution. Ibuprofen release in phosphate buffer, pH 7.4 at 37^0C from the liposomal solution and the liposomal gel were prolonged significantly compared with their respective solution and gel controls. The liposomal gel controlled ibuprofen release and dural permeation *in vitro* and showed a permeation pattern favorable for maintaining constant drug levels. The liposomal poloxamer gel represents a new formulation approach to increase the local epidural availability of ibuprofen. It appeared to be a promising injectable controlled release drug delivery system.[49]

- Choi, *et al.,* studied *in- situ* gelling and mucoadhesive suppository to solve the problems of conventional solid suppositories, which forms gel at body temperature, has a suitable gel strength does not leak out from the anus after administration and has a suitable bioadhesive force so as not to reached the end of the colon. From the results of these studies, it was suggested that the

mixtures of P407/P188 (15/15-15/20) are the optimal systems, which had the gelation temperature suitable for acetaminophen liquid suppository. Furthermore, they suggested that less than 1.0 % of carbopol or polycarbophil must be added to prevent the leakage of the suppositories from the anus and retain the gelled suppositories in the rectum. This system might be applicable for the development of *in situ* gelling and mucoadhesive liquid suppository for humans as a more convenient and effective rectal dosage form.[50]

- Kim, C. K., *et al.,* developed *in-situ* gelling and mucoadhesive acetaminophen liquid suppository using poloxamers and sodium alginate to improve patient's compliance of conventional acetaminophen suppository. Pharmacokinetic study from liquid and conventional solid suppositories in human subjects was carried out. The results showed that acetaminophen liquid suppository with optimal gelation temperature, gel strength and bioadhesive force had a similar release pattern to conventional suppository, which was easy to administer to the anus and showed faster absorption of acetaminophen in human subjects than conventional suppository, was more comfortable for the patients and therefore was thought to be a favorable anti-pyretic and analgesic dosage form for infants and children.[51]

- Elhady, S. S., *et al.,* developed *in situ* gelling and mucoadhesive rectal solution of Mebeverine hydrochloride (MbHCL), which suffers from extensive first pass effect, to improve its bioavailability and possibly restrict its absorption to only the lower rectum. Mixtures of poloxamer 407 and 188 were used to confer the temperature sensitive gelation property. To modulate the gel strength and mucoadhesive force of MbHCL poloxamer rectal solution, mucoadhesive polymers such as HPMC, HEC, methylcellulose and polyvinylpyrrolidone $K_{15}M$ were investigated. The result showed that, these polymers reinforced the gel strength and mucoadhesive force of the prepared solutions. Increasing the concentration of cellulosic bioadhesive polymers retarded the release of MbHCL and it was possible to sustain the drug effect over a period of period of 8 hours.[52]

- *Morimoto, et al.,* showed the nasal absorption of nifedipine from gel preparations in rats. They used PEG 400, aqueous carbopol gel in investigating high bioavailability and prolong action of the drug. Nasal administration of nifedipine in PEG resulted in rapid absorption and high Cmax, however, the elimination of nifedipine from plasma was very rapid. Nifedipine plasma concentration after its nasal administration in aqueous carbopol gel formulation was very low. On the other hand, carbopol-PEG gel containing 50% PEG 400 showed a relatively high nifedipine concentration in the rat blood and prolongs its action.[53]

CH. III .

PLAN OF WORK

- Procurement and characterization of drug sample.
- Interaction studies between drug and polymers.
- Formulation of stable phase transition system.
- Evaluation of phase transition system for
    - Viscosity
    - Mucoadhesion.
    - *In-vitro* release.
    - *In-vitro* permeation studies to evaluate the effect of penetration enhancers in improving permeability through porcine nasal mucosa.
    - Drug content
- Stability studies.

CH. IV. Materials and Methods.

4.1 Materials

Sr. No.	Materials	Supplier
1.	Drug	Cipla Lab. Mumbai.
2.	Chitosan HCl	Mahtani Chitosan Pvt. Ltd. Veraval
3.	Hydroxypropyl Beta Cyclodextrin	Cipla Lab. Mumbai.
4.	Benzalkolium Chloride	Red Cross Formulation, Aurangabad.
5.	Sodium Beta glycerophosphate	Red Cross Formulation, Aurangabad.
6.	Sodium Chloride	--
7.	Distilled Water	--

Note : - *As the work is carried out at commercial organization (Red Cross Formulation, Aurangabad). So the name of pure drug remain confidential and is denoted as " Drug ".*

4.1.1 Instruments

Double Beam UV Spectrophotometer.

Mode No-UV 2401(PC), S.220V.

2. Electronic Weighing Balance

Model No. AW-220.

Shimadzu Corporation, Japan.

3. Electronic Weighing Balance.

Model No. BX 6205.

Shimadzu Asia Pasific Pvt. Ltd.Singapur**.**

4. pH Meter

Model No. (Systronics 361)

5. Brookfeild Viscometer.

Model RV DVG-230

Brookfeild Engg. Lab., Inc., Stoughton.

6. Peristaltic Pump

Model PP201V

Electrolab, Mumbai.

7. Fourier Transmittance Infrared Spectrophotometer.

Model 84005, Shimadzu Asia Pasific Pvt. Ltd.Singapur.

8. Brookfield CAP Viscometers

Models CAP 2000

CH.V

Drug Profile [54,55,57,58,59]

Category : Beta-Adrenoceptor blocker.

Description : White odorless powder

Solubility : Sparingly soluble in water, soluble in ethanol, slightly soluble in dichloromethane practically insoluble in ether.

pKa : 9.6(24^0 C)

Partition coefficient : Log P(octanol/water),0.23

pH : 9.7 (in 1% solution)

Half life : 5-8 hours.

Oral Bioavailability : about 50 %

Melting point :150-1520c

Pharmacopial Status : Official in USP, IP, USP

Pharmacological action:

Drug is a ß1-selective adrenergic antagonist that is devoid of intrinsic sympathomimetic activity and membrane stabilizing property.

Pharmacokinetics:

Absorption:

Drug is incompletely absorbed from the gastrointestinal tract; following oral administration about 50 % is absorbed. Peak plasma concentrations of upto 2 µg/ml are reached in 2-4 hours & has low lipid solubility.

Distribution:

Drug is widely distributed; it crosses the placenta and is excreted in breast milk. Only small amounts are reported to cross the blood brain barriers, plasma protein binding is minimal. Its plasma protein binding is less than 5 %. Its volume of distribution is about 0.5 to 1.5 L/kg

Metabolism:

Only a small part of Drug is metabolized, about 10 % of a dose & is characterized by low hepatic clearance.

Elimination:

Mostly of the drug is eliminated, in its unchanged form, by several routes, but prevailing via the kidney.

Therapeutic uses:

Drug is a cardio selective ß-blocker. It is used in the management of hypertension, cardiac arrhythmias and myocardial infarction. It is also used in the treatment of hyperthyroidism and migraine.

Adverse effects:

Effects on heart: 2.5 mg by intravenous induced atrial fibrillation in 6 of 12 predisposed patient.

Effects on gastrointestinal tract: Reports of sclerosing peritonitis and retroperitoneal fibrosis in a patient taking drug.

Effects on vision: visual sysmptoms without headache were reported with drug for migraine prophylaxis in a patient who had experimented a similar reaction with nadolol.

Effects on lipid metabolism: ß-blockers may increase serum-triglyceride concentrations and there has been a report of acute pancreatitis being provoked by severe hypertriglyceridaemia in a patient in whom ß-blockers greatly impaired triglyceride clearance.

Effect on the Liver: A report of reversible cholestatic hepatitis in a patient receiving drug and hepatic dysfunction.

Interactions:

Catecholamine-depleting drugs (ex. reserpine may have an additive effect when given with ß-blocking agent.

Contraindications:

It is contraindicated in sinus bradycardia, heart block greater than first degree, cardiogenic shock, and overt cardiac failure.

Dose:

Usual adult dose:

Antihypertension: in hypertension, drug is given by mouth in a dose of 50-100 mg daily, as a single dose, although 50 mg daily is generally adequate. The full effect is usually evident within 1 to 2 weeks.

Angina pectoris: The usual dose for angina pectoris is 50-100 mg daily by mouth, given as single or divided doses.

Polymer profile

Chitosan Hydrochloride [60,61,62,63]

Nonproprietary Names **:** Chitosan Hydrochloride,Chitosan Hydrochloridum

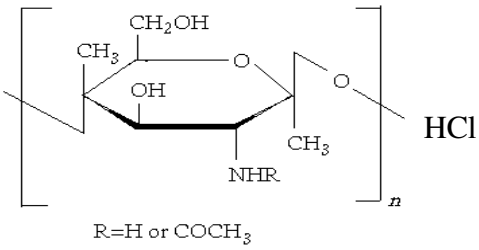
Synonyms **:** Deacetylated chitin; Deacetylchitin

Chemical name **:** Poly-Beta-(1,4)-2-Amino-2-deoxy-Dglucose

Empirical formula molecular weight :

Partial deacetylation of chitin results in the production of chitosan, which is a polysaccharides comprising copolymers of glucosamines and N-acetylglucosamine. Chitosan is the term applied to deacetylated chitins in various stages of deacetylation and depolymerisation and it is therefore not easily defined in terms of its exact chemical composition. A clear nomenclature with respect to the different degrees of Ndeacetylation between chitin and chitosan has not been defined and chitosan is not one chemical entity but varies in composition depending on the manufacturer. In essence, chitosan is chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be greater than 80-85 %. Chitosan is commercially available in several types and grades that vary in molecular weight between 10000 and 1000000,and vary in degree of deacetylation and viscosity.

Structural formula :



Functional category:

Coating agent, disintegrant, film forming agent, mucoadhesive, viscosity-increasing agent.

Description: Chitosan occurs as odorless, white or creamy white powder or flakes.

Solubility : Chitosan hydrochloride is soluble in water.

Solution pH: pH of 1%w/v solution is 4.0-6.0.

Incompatibilities: Chitosan is incompatible with strong oxidizing agents.

Applications in pharmaceutical formulation or technology:

Chitosan is used in cosmetics and is under investigation for use in a number of pharmaceutical formulations. The suitability and performance of chitosan as a component of pharmaceutical formulations for drug delivery applications has been investigated in numerous studies These includes controlled drug delivery application, use as a component of mucoadhesive dosage forms, rapid release dosage forms, improved peptide delivery, colonic drug delivery systems and use for gene delivery. Chitosan has been processed into several pharmaceutical forms including gels films, beads, microsphere, tablets, and coating for liposomes. Further more chitosan may be processed into drug delivery systems using several techniques including spray drying, coacervation, direct compression and conventional granulation processes.

Using chitosan in conventional solid dosage forms:

Chitosans film forming abilities lend itself well as a coating agent for conventional solid dosage forms such as tablets. Furthermore its gel-and matrix forming abilities makes it useful for solid dosage forms such as granules, microparticles, etc. Sakkinen and coworkers studied microcrystalline chitosan as a gel forming excipient for matrix type drug granules. Crystallinity, molecular weight and degree of deacetylation were seen to be factors that affected the release rates from the chitosan based granules .Combination of positively charged chitosan with negatively biomolecules, such as gelatin, alginic acid ,and hyaluronic acid, has been tested to yield novel matrices with unique characteristics for controlled release of drugs.

Site specific targeting:

Tozaki and coworkers utilized chitosan capsules for colon specific delivery totreat ulcerative colitis. A5-amino salicylic acid was encapsulated into chitosan capsules and delivered in vivo to male wistar rats after induction of colitis .it was observed that chitosan capsules disintegrated specifically in the large intestine as compared to the controll formulation (in absence of chitosan) which demonstrated absorption of the

drug in small intestines, this data is a representative example of utility of chitosan for colon specific delivery, while the mechanism of chitosan disintegration is speculative at this point in time, the excipients has promise for site- specific delivery.

Chitosan as permeation enhancer:

It has been reported that chitosan, due to its cationic nature is capable of opening tight junctions in a cell membrane. This property has led to a number of studies to investigate the use of chitosan as a permeation enhancers for hydrophillioc drugs that may otherwise have poor oral bioavailability, such as peptides, because the absorption enhancement is caused by interaction between the cell membrane and positive charges on the polymer, the phenomenon is pH and concentration dependent. Furthermore increasing the charge density on the polymer would lead to higher permeability this has been studied by quaternising the amine functionality on chitosan.

Chitosan as mucoadhesive excipients:

Bioadhesivity is often used as an approach to enhance the residence time of a drug in the gastrointestinal tract, thereby increasing the oral bioavailability. A comparison between chitosan and other commonly used polymeric excipients indicates that the cationic polymer has higher bioadhesivity compared to other natural polymers, such as cellulose, xanthan gum and starch

Stability and storage condition:

Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool, dry place. The Ph.Eur. 2002 specifies that chitosan should be stored at a temperature of 2-8^{0}C.

EXCIPIENTS

Benzalkolium Chloride

Synonym :- Alkyl Dimethyl Benzyl Ammonium Chloride

Molecular Formula :-

$$[\ C_6H_5CH_2N(CH_3)_2\ R]\ Cl.$$

Molecular Weight :- 360.

Description :- It occurs as white or yellowish white amorphous powder, a thick gel or gelatinious flakes. It is hygroscopic, soapy to touch and has mild aromatic odour and has bitter taste.

Functional Category :- Antimicrobial Preservative, Antiseptic, Disinfectant Solubalizing agent, Wetting agent.

Solubility :- Partially insoluble in ether, very soluble in acetone, ethanol, methanol and water.

Stability and Storage :- It is hygroscopic and may be affected by light, air, metals. Solutions are stable over wide Ph and temperature range may be sterilized by autoclaving without loss of effectiveness, solutions may be stores for prolonged period at room temperature.

pH :- 5 to 8 for 10 % w/v aq. soln.

Melting Point :- 40 ^{0}c.

Density :- 0.98 gm/cm^3 at 20 ^{0}c.

Application :- It is used in pharmaceutical formulation as an antimicrobial preservative. In nasal formulation the concentration of 0.002 % to 0.02 % is used. It is also used as preservative in cosmetics.

Hydroxypropyl Beta Cyclodextrin

Synonym :- Beta Cyclodextrin 2-hydroxypropyl ether**.**

Molecular Formula :-

$C_{22}H_{32}N_2O_2$

Molecular Weight :- 356.50.

Description :- It is water soluble, free flowing, white odorless powder. It is produced from Beta Cyclodextrin by hydroxylpropylation of hydroxyl groups of the cyclodextrin.

Functional Category:- Suitable for molecular encapsulation of variety of sparingly water soluble compounds to enhance the aqueous solubility of the encapsulated compounds.

Solubility :- 45 % (w/v) aq 2-hydroxypropyl-β-cyclodextrin.

Storage Temperature :- 2 to 8 ^{0}C.

Optical Activity :- [α] /D + 7.8^0, c = 0.825 in methanol(lit).

Application :- It is used for solubalization and stabilization of guests. By complexing with HPBCD, the guest interacts with cavity of HPBCD to become entrapped. The outer surface of the HPBCD is very hydrophilic and interacts well with water to carry the guest into the solution. Some proteins can also be solubalized with HPBCD.

Sodium Beta glycerophosphate

Synonym :- 2- glycerophosphate Disodium Salt 5-H_20

Molecular Formula :-

$C_3H_{17}Na_2O_{11}P$

Molecular Weight :- 306.11.

Description :- It is water soluble, free flowing, white odorless powder.

Functional Category :- Induces hydrogel formation when placed at physiological temperature.

Solubility : - Water Soluble 0.1 gm/ml clear, colourless.

Storage Temp :- 2 to 8 ^{0}c**.**

Melting Point :- 102 to 104 ^{0}c

Application : - It is use for the preparation of temp. triggered phase transition system.

Sodium Chloride

Synonym :- Common Salt, Table Salt, Sea Salt.

Molecular Formula :- NaCl.

Molecular Weight :- 58.44.

Description :-

NaCl occurs as white crystalline powder or colourless crystals, it has saline taste. The crystal lattice is face-centered cubic structure. It contents no water or crystallization although below 0 ^{0}c. Salt may crystallize as dehydrate.

Functional Category:- Isotonicity modifier for nasal and opthalmic formulation.

Solubility : - Water Soluble

Storage :- At room temp.

pH :- 6.7 – 7.3

Boiling Point :-141 ^{0}c

Applications :- NaCl is widely used in variety of parentral and non parentral formulation. It is used as isotinicity modifier.

CH. VI.

EXPERIMENTAL INVESTIGATION.

Preliminary Study

Characterization of Drug.[55,57,59]

Drug was characterized according to I.P. and USP.

Description:

- **Nature** : Amorphous powder
- **Color** : White
- **Odor** : Odorless
- **Solubility** : Sparingly soluble in water.
- **pH** : 9.7, determined in a1% w/v solution
- **Melting point :** 150-152 ^{0}c.
- **Identification:**
- **UV absorption spectroscopy**

Drug solution of 100 µg/ml in phosphate buffer saline pH 7.4 was scanned in the range of 200- 400 nm.

The λmax for drug was found to be 274 nm as shown in Figure 8**.**

Scanning of λmax of drug by UV spectrophotometry:

Scanning was done in phosphate buffer saline (PBS) pH 7.4 (disodium hydrogen phosphate 2.38 g, potassium dihydrogen phosphate 0.19 g and sodium chloride 8.0 g for 1000 ml). A solution of 100 µg/ml drug was prepared and UV spectrum was recorded in the range of 200-400 nm.

Characterization of Chitosan Hydrochloride

pH determination[45,63]

: 1 % w/v solution of chitosan hydrochloride solution in water was made and pH of solution was measured using pH meter.

Observation : pH was found to be 5.4(reported 4.0- 6.0).

Gelation study :

1 % w/v of chitosan HCl was made and neutralized with 1 molar NaOH.

Observation : Above pH 6.2 chitosan HCl forms hydrated gel like structure .

Drug Polymer Interaction Study

Drug polymer interaction studies were carried out by two methods

1) UV Spectrophotometry
2) FTIR Spectroscopy

UV spectrophotometry [42]

In order to see drug polymer interaction, 100 µg/ml of pure drug solution, chitosan hydrochloride solution, chitosan hydrochloride solution containing drug, were scanned for UV absorption between 200 to 400 nm. UV scans of polymeric solutions containing drug were compared with those of pure drug solution. UV spectra shown in Figure11

FTIR Spectroscopy [42,56,57,65]

To see drug polymer interaction FTIR studies were carried out. Pure drug, pure chitosan hydrochloride, physical mixture of drug and chitosan hydrochloride was analyzed using KBr disk method. FTIR spectra are shown in Figure 12.

Standard curve of drug : Standard curve of a drug was prepared in phosphate buffer saline pH 7.4 Drug was dissolved in phosphate buffer saline pH 7.4 to get the stock of concentration 1000 µg/ml. From this stock solution serial dilution was done to get drug concentration of 10 to 100 µg/ml. The absorbance of the solutions was measured against PBS 7.4 as a blank at 274 nm, using double beam UV- visible spectrophotometer. The graph of absorbance v/s concentration is shown in Figure 13.

The absorbance's of drug are shown in Table 3. The standard calibration curve is depicted in Figure 13.

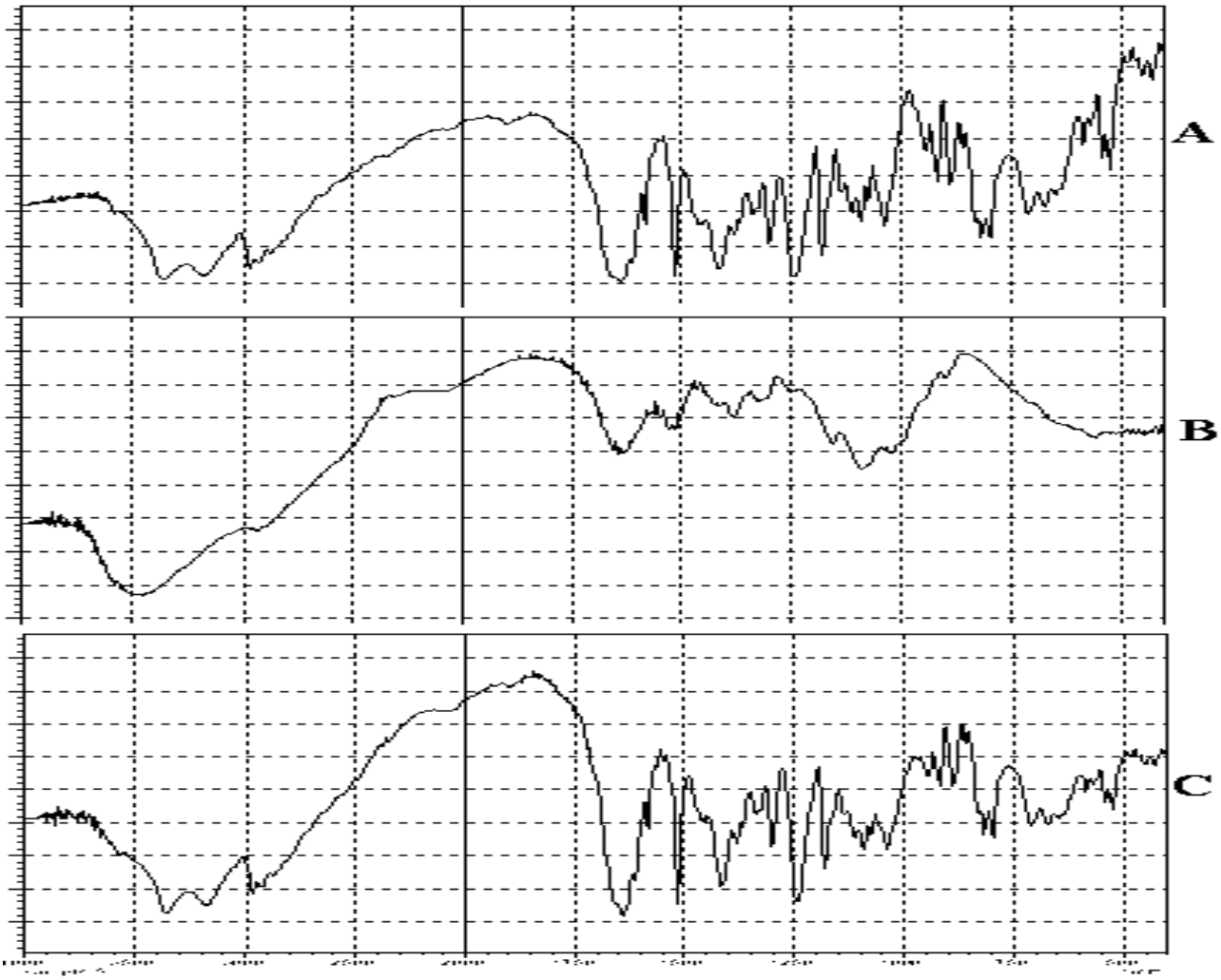
Dose Calculation[54,5]

5

Oral dose of Drug is 50-100 mg daily in one or two doses.

I. V. injection in a dose of 2.5 mg at a rate of 1 mg / min is given with a total dose 10 mg.

Oral bioavailability is only 50 % due to low lipid solubility.



Not more than 5 % w/w of HP β cyclodextrin is recommended for nasal administration, therefore molar ratio 1:0.19 was selected considering safety and this concentration is sufficient to give the solubility sufficient enough to incorporate the dose (20 mg) in 0.4 ml for both nostril of nasal solution.

Table 4 : **Solubility of drug in water with different ratios of HP β CD**

Molar ratio of Drug: HP β CD	**Solubility of Drug (mg/ml)**
1:0	16
1:0.19	60
1:0.50	95
1:1	140

Formulation of Phase Transition System

The formulation 0

● A separate solution of drug with hydroxy propyl β-cyclodextrin was prepared.

● The formulation 0 was prepared without adding chitosan hydrochloride and sodium β - glycerophosphate.

●Sodium chloride was added to maintain isotonicity.

●Finally benzalkonium chloride was added and the volume was make up with distilled water.

●The final pH of the formulation was adjusted to 6.8.

●Each ingredient was separately sterilised by autoclaving at 121^0C and 15 Psig for 20 minutes and added aseptically in laminar air flow work station.

Table 5 : Composition of drug phase transition systems

Sr no	ingredients	Quantity taken
1	Drug (g)	5
2	HP-β-CD (g)	5
3	Sodium Chloride (g)	0.5
4	Benzalkonium Chloride (ml)	0.02
5	Distilled Water (ml)	Upto 100

Formulation 1

●

The formulation 1 using 1 % chitosan hydrochloride as mentioned in the table was prepared by dissolving the chitosan hydrochloride in the distilled water and cooling to ~4^0 C.

● The pH was lowered by 1M HCl To this solution sodium β-glycerophosphate solution was added drop by drop with continuous stirring.

● To the chitosan hydrochloride/glycerophosphate solution a separately prepared solution of drug with hydroxy propyl β-cyclodextrin was added. .

● Sodium chloride was added to maintain isotonicity.

● Finally benzalkonium chloride was added and the volume was make up with distilled water.

● The final pH of the formulation was adjusted to 6.8.

● Each ingredient was separately sterilised by autoclaving at 121^0C and 15 Psig for 20 minutes and added aseptically in laminar air flow work station.

Table 6 : Composition of drug phase transition systems using Chitosan hydrochloride

Sr no	ingredients	Quantity taken
1	Drug (g)	5
2	HP-β-CD (g)	5
3	Chitosan Hydrochloride (%W/V)	**1**
4	Sodium β Glycerophosphate (g)	**4.12**
5	Sodium Chloride (g)	0.5
6	Benzalkonium Chloride (ml)	0.02
7	Distilled Water (ml)	Upto 100

Formulation 2

●

The formulation 2 using 2 % chitosan hydrochloride as mentioned in the table was prepared by dissolving the chitosan hydrochloride in the distilled water and cooling to ~4^0 C.

● The pH was lowered by 1M HCl To this solution sodium β-glycerophosphate solution was added drop by drop with continuous stirring.

● To the chitosan hydrochloride/glycerophosphate solution a separately prepared solution of drug with hydroxy propyl β-cyclodextrin was added. .

● Sodium chloride was added to maintain isotonicity. Finally benzalkonium chloride was added and the volume was make up with distilled water.

● The final pH of the formulation was adjusted to 6.8.

● Each ingredient was separately sterilised by autoclaving at 121^0C and 15 Psig for 20 minutes and added aseptically in laminar air flow work station.

Table 7 : **Composition of Formulation2 phase transition systems using Chitosan hydrochloride**

Sr no	ingredients	Quantity taken
1	Drug (g)	5
2	HP-β-CD (g)	5
3	Chitosan Hydrochloride (%W/V)	2
4	Sodium β Glycerophosphate (g)	8.25
5	Sodium Chloride (g)	0.5
6	Benzalkonium Chloride (ml)	0.02
7	Distilled Water (ml)	Upto 100

Evaluation of Formulations

Gelation Temperature [70,71]

The gelation temperature was estimated by heating the solution (about 1-2^{0}C/min) in a test tube with gentle stirring until gel formed. Gel formation was taken as the point when there was no flow, when the container was overturned. The temperature was noted and designated as gel formation temperature.

Three replicates of each measurements was done and results were reproducible within the range. Stirring speed and heating - cooling rates have no significant effect on the transition temperature

Viscosity Measurements [42,70,71,72,73,74]

For the measurements of viscosity, Brookfield CAP Viscometer was used. 1 to 2 drops of sample was placed on the temperature sensitive plate. All the parameters on the instruments were fixed as per the instruction manual, then after some time the set temperature was achieved. Cone No. 3 was hold on the plate and the viscosity readings were taken from the display of the instrument.

Rheological Behavior [75]

To determine the type of flow, Brookfield CAP Viscometer was used. 1 to 2 drops of sample was placed on the temperature sensitive plate. All the parameters on the instruments were fixed as per the instruction manual, then after some time the set temperature, 37^{0}C was achieved. Cone No. 3 was hold on the plate and the shear stress and shear strain values were taken from the display of the instrument. The rheograms of Formulations 1 and 2 were plotted.

Mucoadhesion Study [51,52,71,76,77,78]

The bio- and mucoadhesion methods found in the literature are based on measuring the force required to break the adhesive bond between the model membrane and adhesive. Mucoadhesive force of nasal phase transition system was determined by means of the mucoadhesive force measuring device shown in **Figure 16** and according to method adopted by (Yong, *et al.* 2001and Elhady, *et al.* 2003), using porcine nasal mucosa and phosphate buffer saline as the moistening fluid. At the time of testing, a section of tissue was secured, keeping the mucosal side out, on to each glass vial using a rubber band and an aluminium cap. One vial with a section of tissue was connected to the balance and other vial was kept in petridish. To the exposed tissue on this vial, a constant amount of 0.5 gm polymer gel was applied. The height of the vial was adjusted so that the gel could adhere to the mucosal tissues of both vials. A constant force was placed on the upper vial and applied for two minutes, after which it was removed and weights were added at a constant rate to the pan on the other side of the modified balance of the used device until the two vials were separated. The Mucoadhesive force, expressed as the detachment stress in dynes/cm^2 was determined from the minimal weights that detached the two vials using the following equation.

$$\text{Detachment stress (Dynes/cm}^2\text{)} = \frac{m.g}{A}$$

Where,

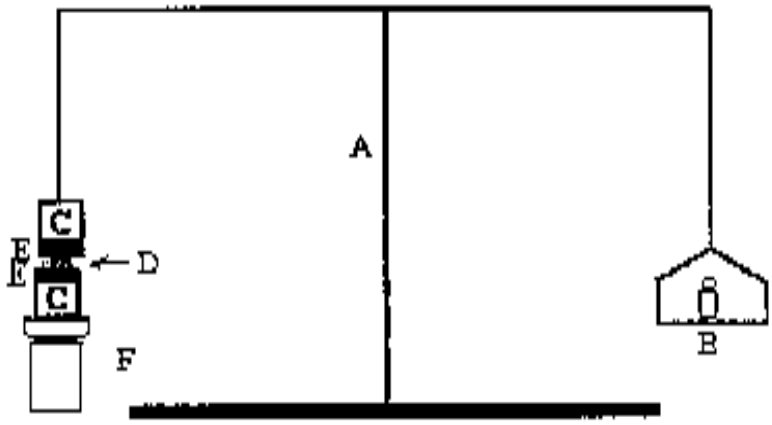
m: The weight added to balance in grams.

g: Acceleration due to gravity taken as 980 cm/ sec^2

A: Area of the tissue exposed and is equal to πr^2

(r- The radius of the circular hole in the aluminium cap).

Figure 16: Mucoadhesive force measuring device, A-modified balance B-weights, C- glass vial, D- polymer gel, E-porcine nasal mucosa, F- support to vial to adjust height.



Duration of Mucoadhesion [71,79,80,81]

This is an important factor in the formulation of bioadhesive dosage forms capable of being retained on mucosal surfaces for extended period of time and must be given careful consideration. Some test systems were designed to assess the effect of various applied forces on the duration of mucoadhesion of the formulation 1 and 2, and to consider the duration of adhesion with regard to the adhesive strength of these materials.

Method 1: This parameter was determined by a method modified from that of suzuki et. al. (1985). An agar plate (containing agar at 1.5%w/v) of 7 cm in diameter was prepared with pH 7.4 phosphate buffers. Five mg of the sample was placed on the center of the agar plate and a circle 5 mm in diameter was made. The plate was slanted at 30^0 and the distance moved by the sample at 23^0 C was measured.

Method 2: Duration of mucoadhesion of phase transition system was determined by means of the mucoadhesive force measuring device (Figure16) and according to previously reported methods (Yong *et.al*. 2001 and Elhady, *et al.* 2003), using porcine nasal mucosa and phosphate buffer saline as the moistening fluid. At the time of testing a section of tissue (E) was secured, keeping the mucosal side out, onto each glass vial(c) using a rubber band and an Aluminium cap. One vial with a section of tissue was connected to the balance (A) and other vial was kept in petridish (F). To the exposed tissue on this vial a constant amount of 0.5 g polymer gel (D) was applied .The height of the vial was adjusted so that the gel could adhere to the mucosal tissues of both vials. A constant force was placed on the upper vial and applied for two minutes, after which it was removed and weight of 10 g was added to the pan on the other side of the modified balance of the used device until the two vials were separated. Duration of mucoadhesion was noted as the time in hours taken to separate two vials.

In- Vitro Release Study [36,42,72,74,82]

In-vitro release study of the formulated phase transition system were carried out in two chamber diffusion cell through dialysis membrane-70 (Himedia) with molecular weight cut off 12000-14000 kDa. Diffusion cell of diameter 1.1 cm and 22 ml capacity consisted of upper cylindrical compartment open from above and diffusion membrane at its base. To prepare artificial membrane, pieces of dialysis membrane were soaked in phosphate buffer saline pH 7.4 for 24 hours before mounting on a diffusion cell.

Diffusion membranes were mounted in a two chamber cells at 30 ^{0}C. Phase transition system of chitosan hydrochloride loaded with drug were placed in the donor compartment. PBS 7.4, 100 ml was placed in the receptor compartment. The temperature of the receiver was maintained at 37 ^{0}C ± 1.0 ^{0}C during the experiment. An aliquot of 1 ml was withdrawn from receiver compartment initially after 5, 15 and 30 minutes and then at 1-hour interval and replaced with same amount of medium. Aliquots so withdrawn were suitably diluted and analyzed using UV spectrophotometer at 274 nm for drug. *In-vitro* release study was carried out for 4 hours**.** Table 21 shows the data of release profile of drug from phase transition system of chitosan hydrochloride and Figure 17 shows release profile of drug from phase transition system of chitosan hydrochloride.

1) **Conc** = absorbance/slope

2) **% release =** amount release/ label claim x 100

In-Vitro Permeation Study [4,36,44,62,68,83,84]

Tissue model

Excised nasal mucosa of different species are frequently used tools to study nasal transport and metabolism. Taking the difficulties into account to obtain human tissues from nasal biopsies, it become obvious that most studies were performed with epithelia excise from animals. For majority of studies rabbit tissue has been used. In this study porcine nasal mucosa obtained from local slaughterhouse has been chosen as model membrane, mainly because of ready and economic availability in sufficient quantity and reproducible quality

Tissue preparation

The skin around the nasal region was removed and snout was separated from the animal and opened up to expose the conchae. The mucosa covering the ventral nasal conchae (cavity mucosa) was carefully removed using forceps and a scalpel. After being rinsed in saline solution and then distilled water, a piece of nasal mucosa was mounted as flat sheet in a two chamber diffusion cells at 37 ^{0}C. Formulations were placed on mucosal surface in the donor compartment. Phosphate buffer pH 7.4 was placed in receptor compartment. The temperature of receiver compartment was maintained at 37 ^{0}C ± 1 ^{0}C, during the experiment.

An aliquot of 1 ml was withdrawn from receiver compartment initially after 5, 15 and 30 minutes and then at 1-hour interval and replaced with same amount of PBS 7.4. Aliquots so withdrawn were suitably diluted and analyzed using UV spectrophotometer at 274 nm for drug. The *in vitro* permeation study was carried out for 4 hours. Data showing *in- vitro* permeation profile of drug from different formulation through nasal membrane is mentioned in Table 26 and Figure 18, 19**.** The permeability coefficient (P) was calculated using the following equation and values are shown in Table 27

$$P = \frac{dQ/dt}{Co.A}$$

Where:

dQ/dt : Permeability rate

Co: Initial conc. in the donor chamber.

A: Effective surface area of mucosa.

Drug Content [42,65,69]

1 ml of the preparation was transferred to 1000 ml volumetric flasks with a pipette and final volume was made up with phosphate buffer saline pH 7.4 absorbance was recorded at 274 nm. The results for drug content was determined by using formula,

% Drug content = absorbance x dilution factor x label claim x 100.

It contains not less than 90.0%and not more than 110.0% of the labeled amount of pure drug.

Stability Study [88,89,90]

Stability study was performed on formulation 1 and 2. The formulations were stored at 10 ^{0}C ± 2 ^{0}C and 75% ± 5% Rh, 25 ^{0}C ± 2 ^{0}C and 75 % ± 5 % Rh and 40 ^{0}C ± 2 ^{0}C and 75 % ± 5 % Rh, for a period of 3 months. Formulations were evaluated at periodic intervals for drug content, viscosity and gelation temperature. Data for stability study is shown in Table 15 and 16.

CH. VII.

RESULTS AND DISCUSSION.

The drug sample was characterized on the basis of physicochemical and spectral analysis to examine its authenticity. The results confirmed it to be the pure sample of drug.

The polymer chitosan hydrochloride was also characterized on the basis of identification tests and confirmed to the reported values.

Scanning of λmax of drug by UV spectrophotometer

Scanning of drug by UV spectrophotometer showed λmax 274 nm and the standard curve at 274 nm followed Beer-Lambert's Law in the concentration ranging from 0-60 µg/ml.

Drug polymer interaction study

Interaction studies were performed by UV scanning and FTIR spectroscopy. No change in λmax of pure drug and drug containing polymeric solution was observed. Drug free polymeric solution showed no absorption at 274 nm.

IR spectra of drug free polymer showed no matching peaks with the drug. The characteristic peaks of the drug (1633,1245,1510,1184,805,820 cm^{-1}) was found same in the IR of the physical mixture of drug and chitosan hydrochloride.

Solubility of drug

Hydroxy propyl β-cyclodextrin was used to increase the solubility of drug in water because of its property to form the inclusion complexes with a guest which results in increased solubility of the drug.

Formulation of phase transition system

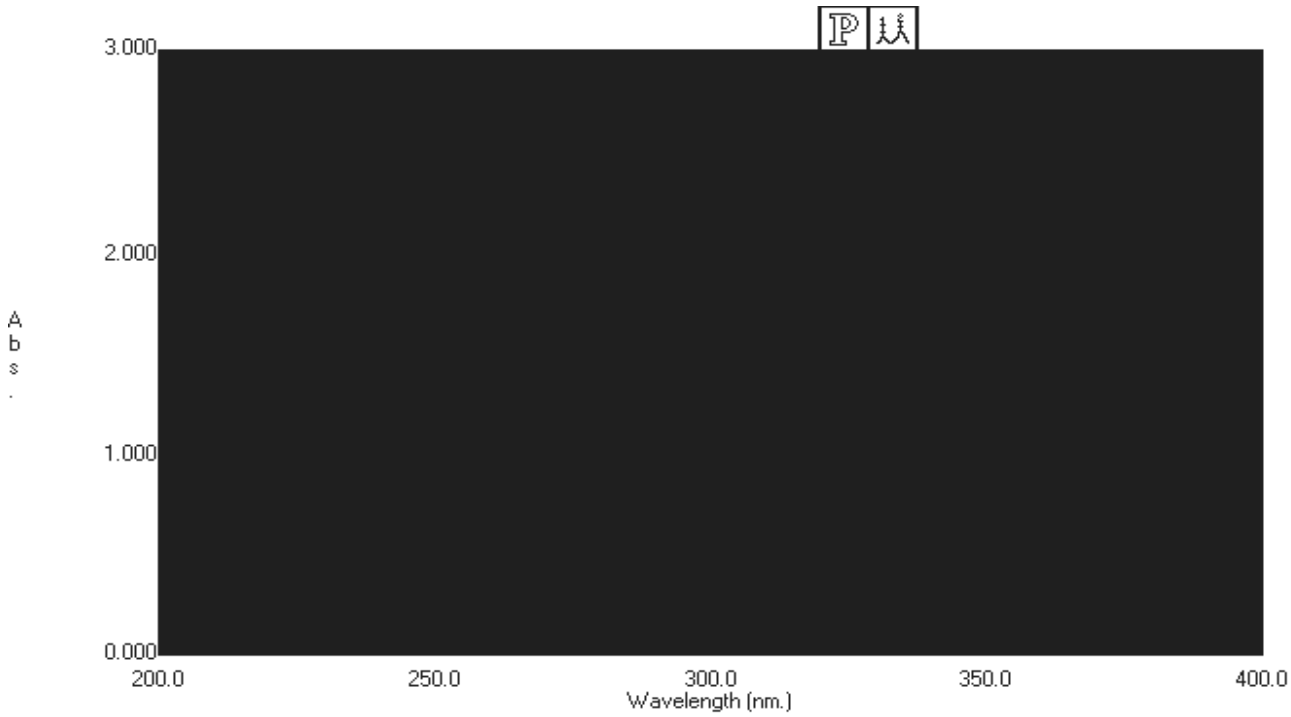
For the formulation of phase transition system chitosan hydrochloride and glycerophosphate was used to prepare temperature triggered phase transition system. Chitosan hydrochloride and glycerophosphate solution induces hydrogel formation when placed at physiological temperature. Chitosan hydrochloride precipitates to form hydrated gel above pH 6.5.When drug is added it increases the pH of the

solution and induces precipitation even before addition of complete addition of drug. Thus temperature induced precipitation was aimed by adding beta glycerophosphate, which keeps the chitosan in hydrated, solution form by getting incorporated between the chitosan molecules and avoiding hydrophobic interaction at lower temperature, dehydration at higher temperature leads to geletion.

TABLES AND FIGURES.

Identification of drug :UV absorption spectroscopy

Figure8: UV Spectra of absorbance maxima of Drug in PBS 7.4



Infra-red absorption spectroscopy:

Figure9: Reference FTIR spectra of Drug

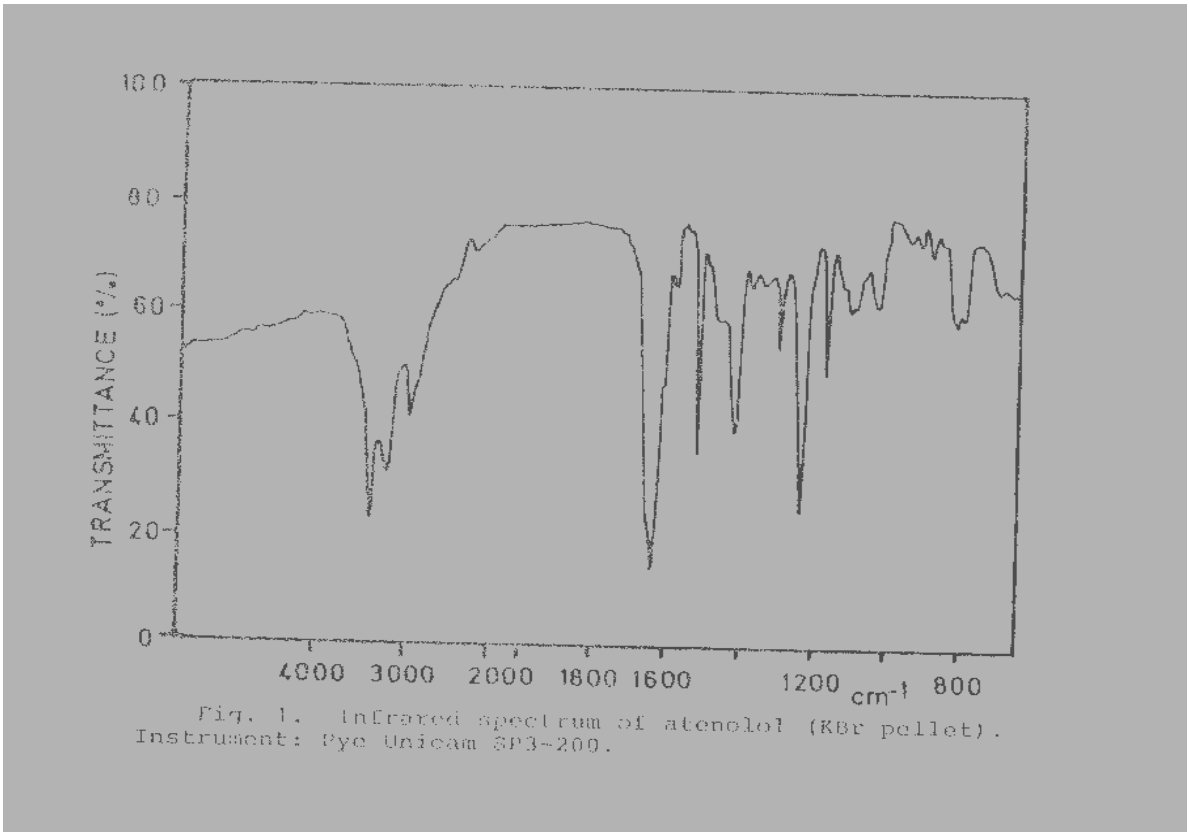
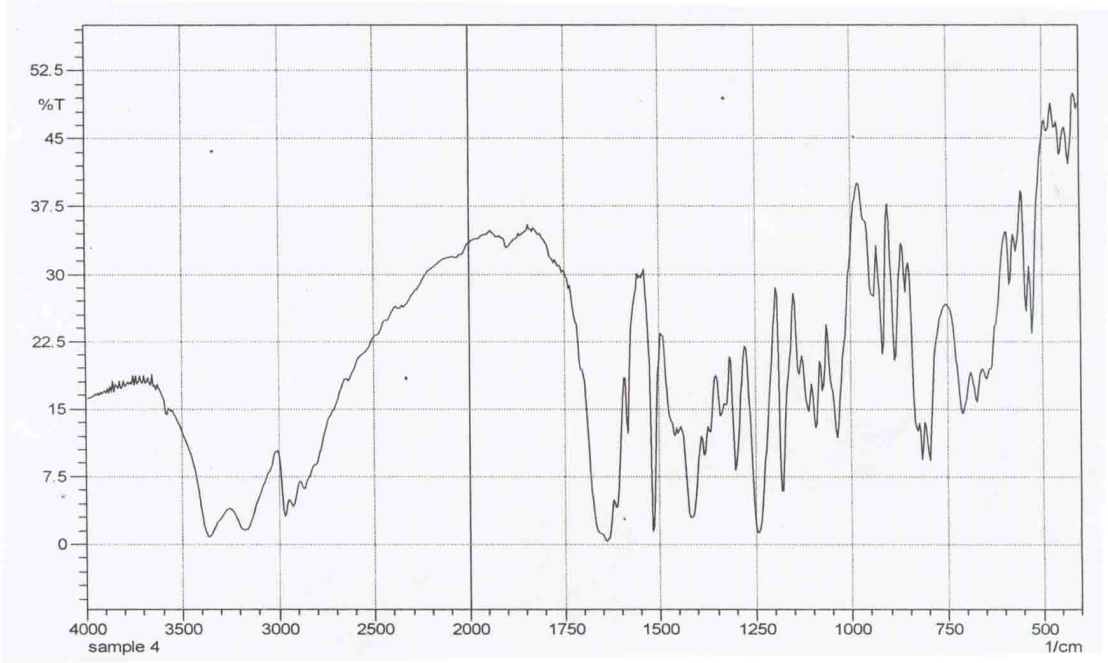


Figure 10 : FTIR spectra of Drug sample

4.2. Analytical Methodology [56,57,64]



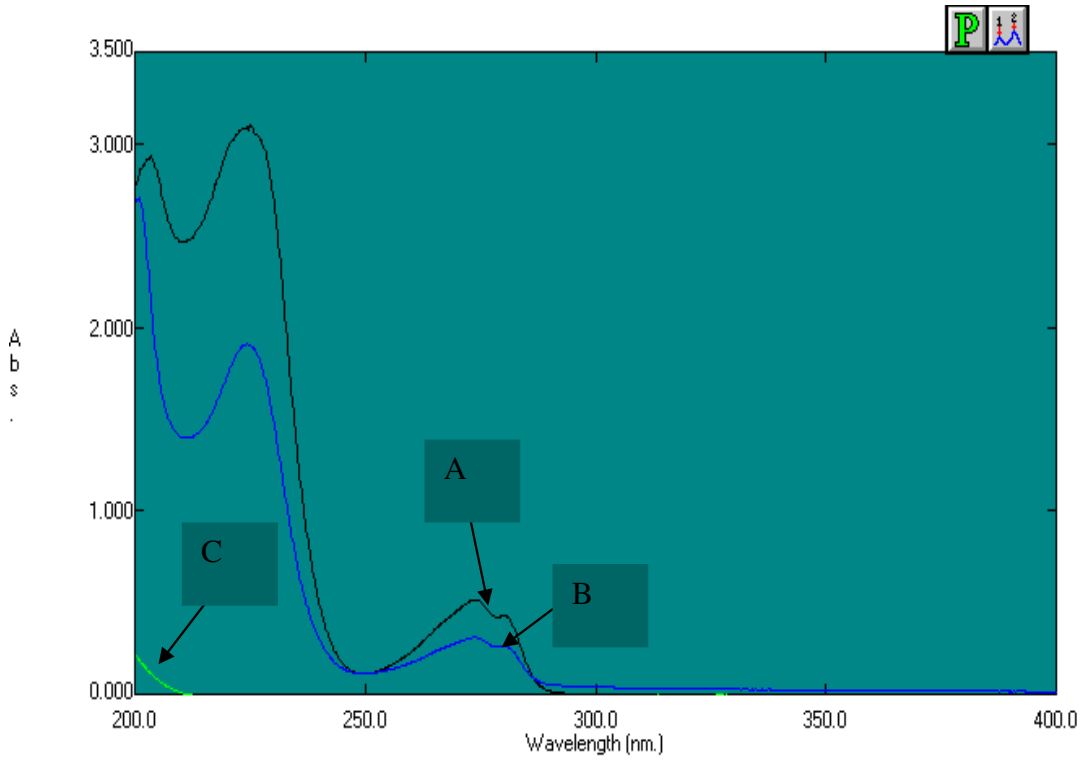
Observation :FTIR spectrum of drug was taken as shown in Figure10.

The Characteristics peaks of the drug was found similar to that mentioned for the reference spectra

Drug Polymer Interaction Study

UV spectroscopy

Figure 11 :-UV spectra of drug A;Chitosan hydrochlofide soln;and Chitosan hydrochlofide soln + drug

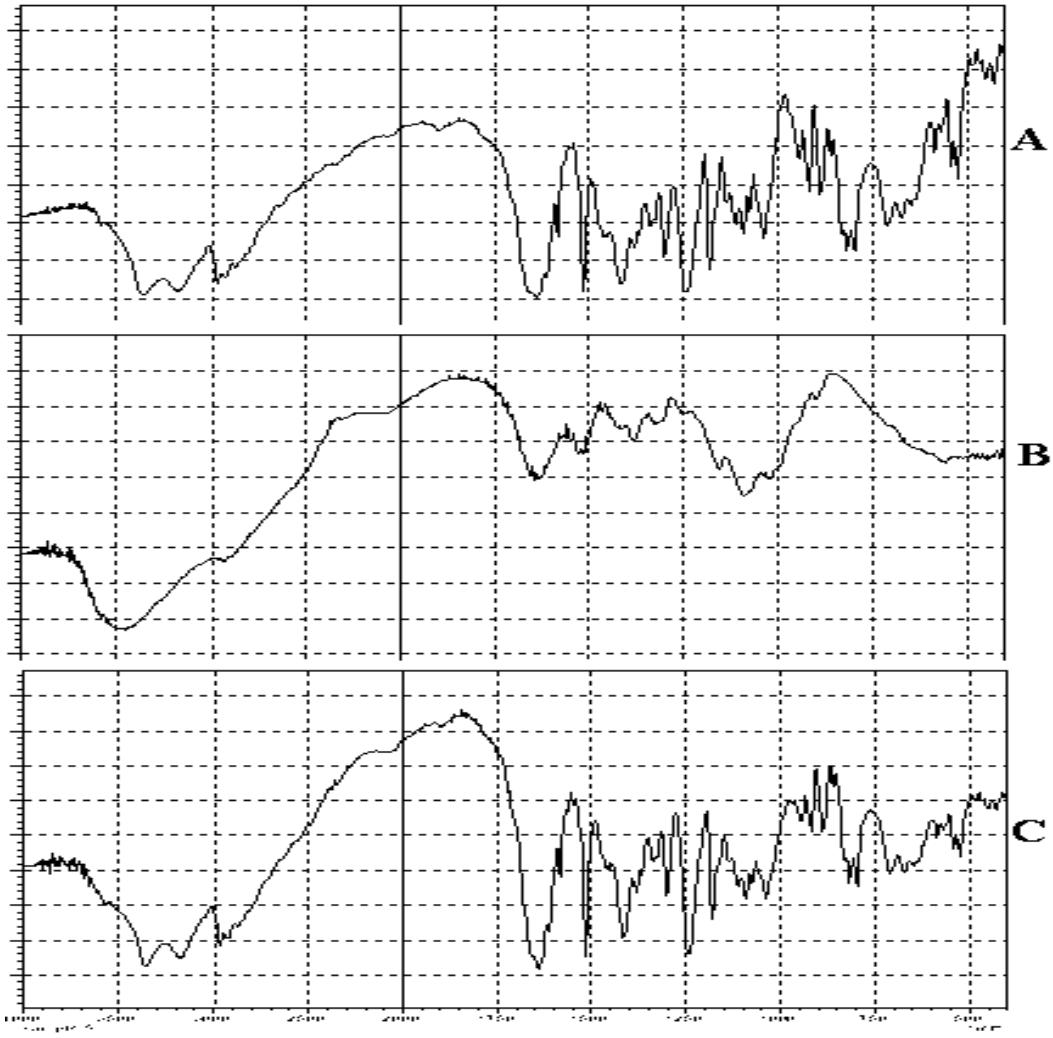


FTIR Spectroscopy

Figure 12 : FTIR Spectra of A : drug,

B: Chitosan hydrochloride,

C : Physical Mixture of Drug and Chitosan hydrochloride

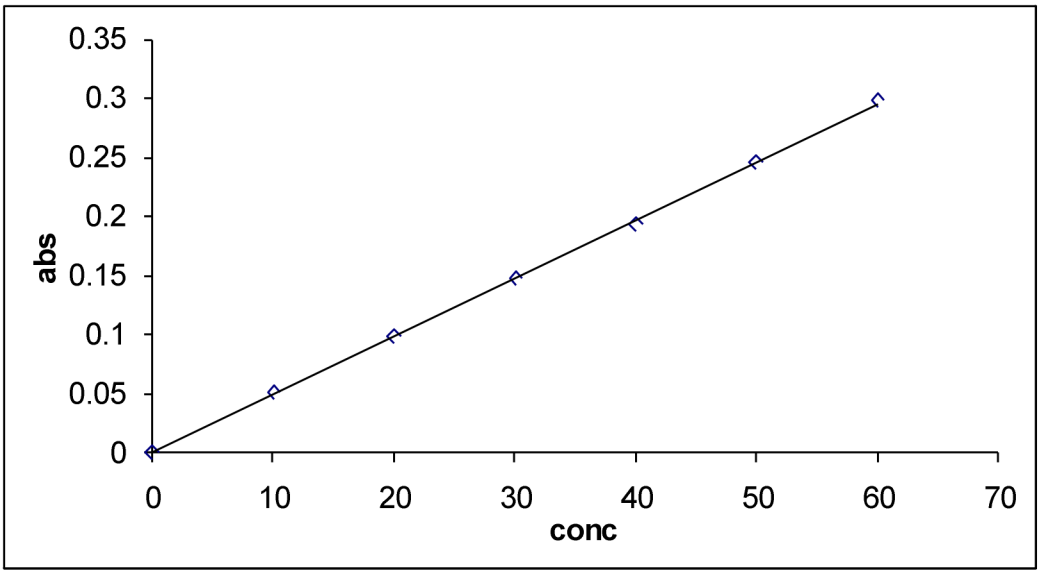


Standard curve of drug

Table 3 : Absorbance values of drug in PBS 7.4 at 274 nm.

Sr no	conc.(µg/ml)	Absorbance(nm)
1	10	0.05
2	20	0.098
3	30	0.147
4	40	0.193
5	50	0.245
6	60	0.298

Figure 13 : Standard calibration curve of drug in PBS 7.4 at 274 nm.



EVALUATION OF FORMULATION.

The developed formulations were evaluated for percent drug content, viscosity, Mucoadhesion, duration of mucoadhesion, *in vitro* release, *in vitro* permeation,

Gelation temperature

Table 8: Gelation temperature of Formulations 1 and 2

Sr. No	Formulations	Gelation Temperature (^{0}C)
1	1	35-37
2	2	34-37

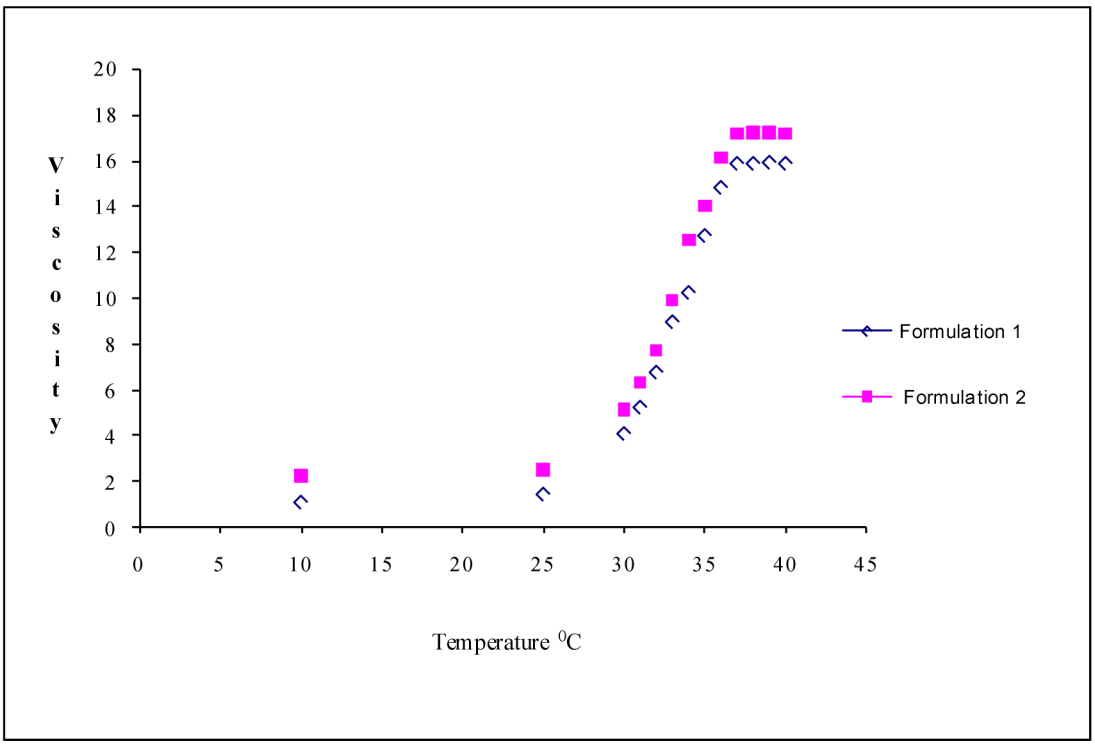
Viscosity Measurments

Table 9 : Viscosity of the Phase Transition System under increasing temperature

Temperature (^{0}C)	Viscosity (poise)	
	Formulation 1	Formulation 2
10	1.05	2.20
25	1.40	2.46
30	4.05	5.12
31	5.23	6.30
32	6.73	7.70
33	8.93	9.89
34	10.21	12.54
35	12.70	14.05
36	14.80	16.15
37	15.85	17.20
38	15.85	17.25
39	15.90	17.26
40	15.85	17.20

Figure 14 :

Viscosity of the Phase Transition System under increasing temperature



Rheological Behaviour

Figure 15A : Rheogram of Formulation 1

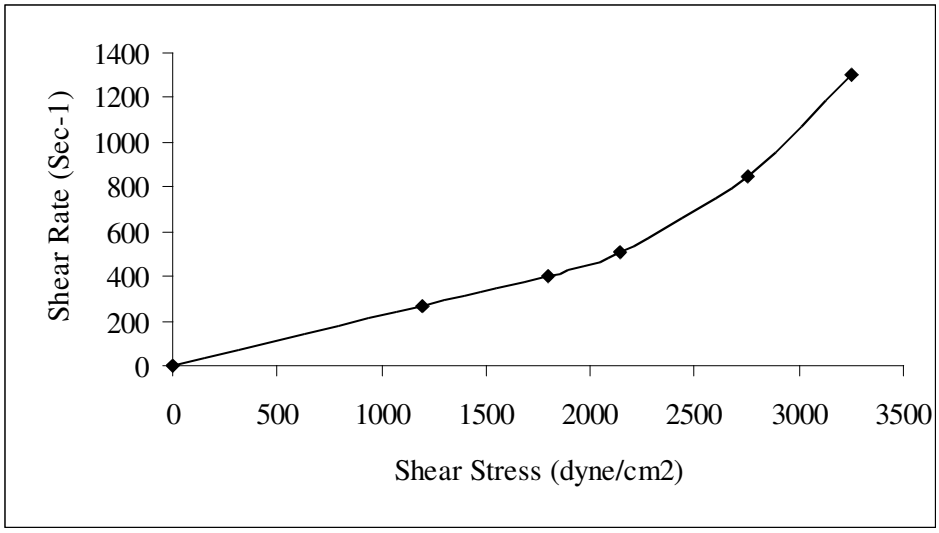
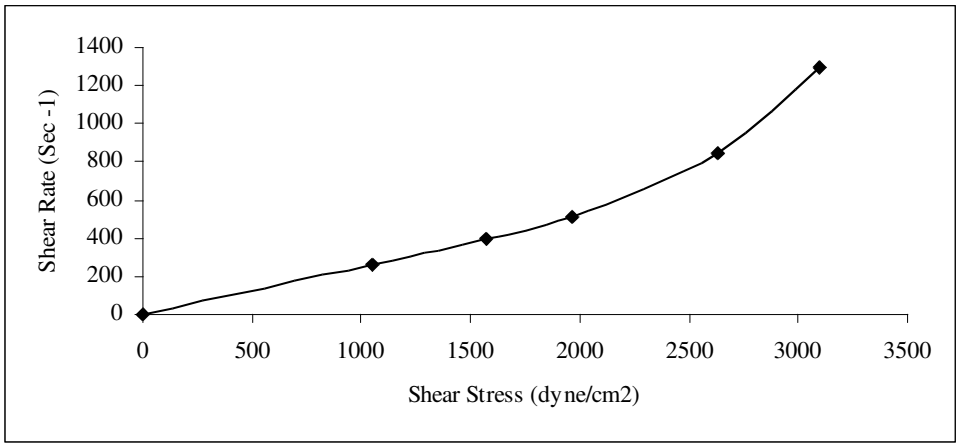


Figure 15B: Rheogram of Formulation 2



Mucoadhesion Study

Table 14 : Mucoadhesive force of chitosan hydrochloride phase transition system

Sr. No	Formulations	Mucoadhesive force (dynes/cm^2 x 10^2)
1	1	24.63 ± 0.36
2	2	32.39 ± 0.42

Duration of Mucoadhesion

Table 15 : Movement of Formulation on Agar plate in cms.(method 1)

Formulations	Time (Hr)					
	1	**2**	**3**	**4**	**5**	**6**
1	0.4	1.0	1.7	2.5	2.8	>3
2	0.2	0.6	1.2	1.9	2.2	2.7

Table 16 : Duration of Mucoadhesion by Method 2

Sr. No	Formulations	Time (Hr)
1	1	4.05
2	2	4.30

In vitro release study

Table 17: Data showing release profile of plain drug solution through dialysis membrane

Time(mins)	Absorbance(nm)	conc.(µg/ml)	% release
5	0.012	0.24	1.2
15	0.0206	0.412	2.06
30	0.030	0.600	3.00
40	0.045	0.90	4.5
120	0.0648	1.296	6.48

180	0.0993	1.986	9.93
240	0.1047	2.094	10.47

Figure:17

Graph showing the release profile of plain drug solution through dialysis membrane

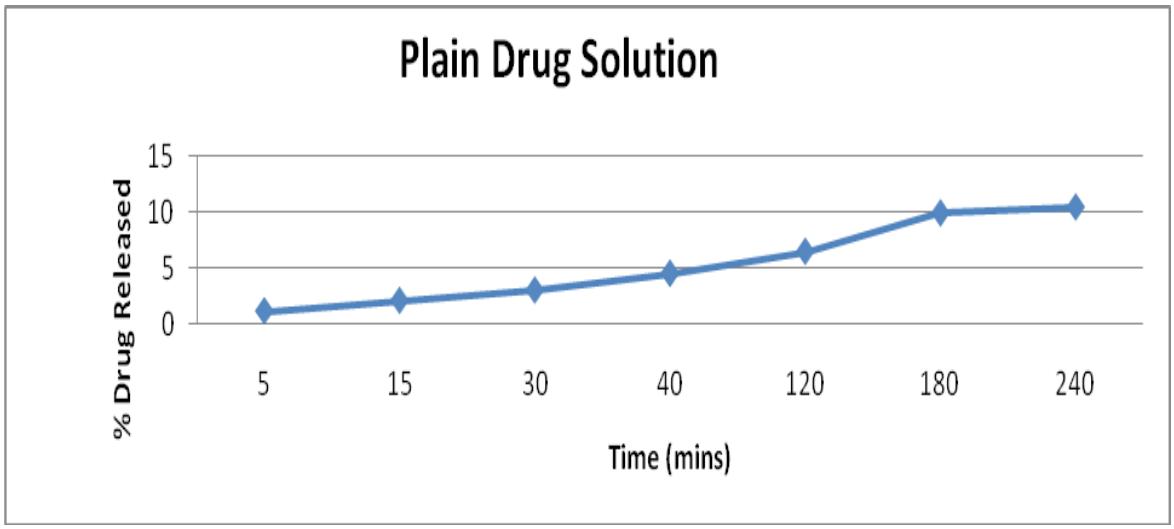


Table18 : **Data showing release profile Formulation 0 through dialysis membrane**

Time(mins)	Absorbance(nm)	conc.(µg/ml)	%release
5	0.0246	0.492	2.46
15	0.0436	0.872	4.36
30	0.0586	1.172	5.86±
40	0.0925	1.85	9.25
120	0.1364	2.728	13.64
180	0.1913	3.826	19.13

240	0.2307	4.614	23.07

Figure 18 :

Graph showing the release profile of formulation 0 through dialysis membrane

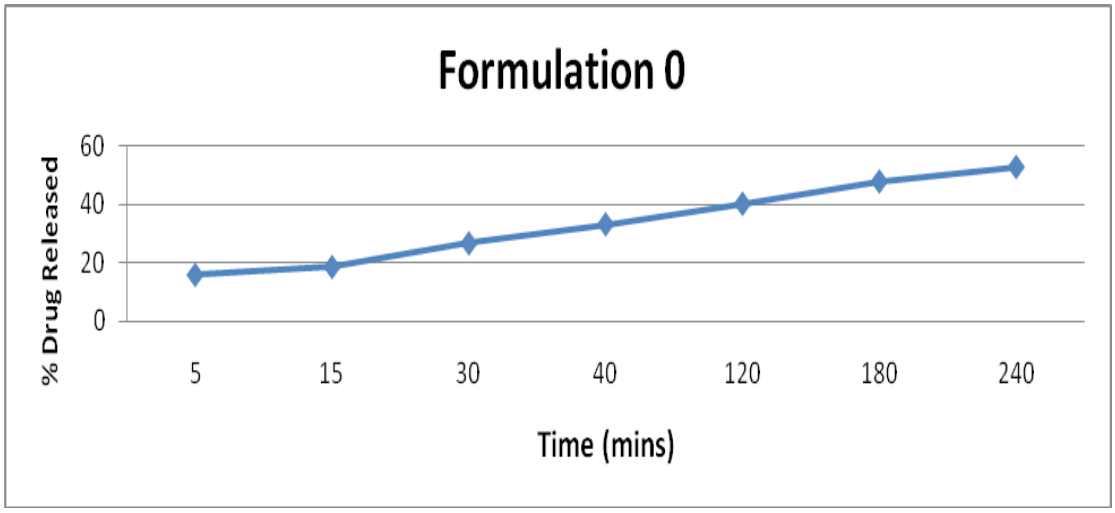


Table19 : Data showing release profile Formulation 1through dialysis membrane

Time(mins)	Absorbance(nm)	conc.(µg/ml)	%release
5	0.751	10502	7.51
15	0.1282	2.564	12.82
30	0.1884	3.768	18.84
40	0.2466	4.932	24.66
120	0.3023	6.046	30.23
180	0.3644	7.292	36.46
240	0.4020	8.04	40.20

Figure 19 :

Graph showing the release profile of formulation 1through dialysis membrane from phase transition system of chitosan hydrochloride.

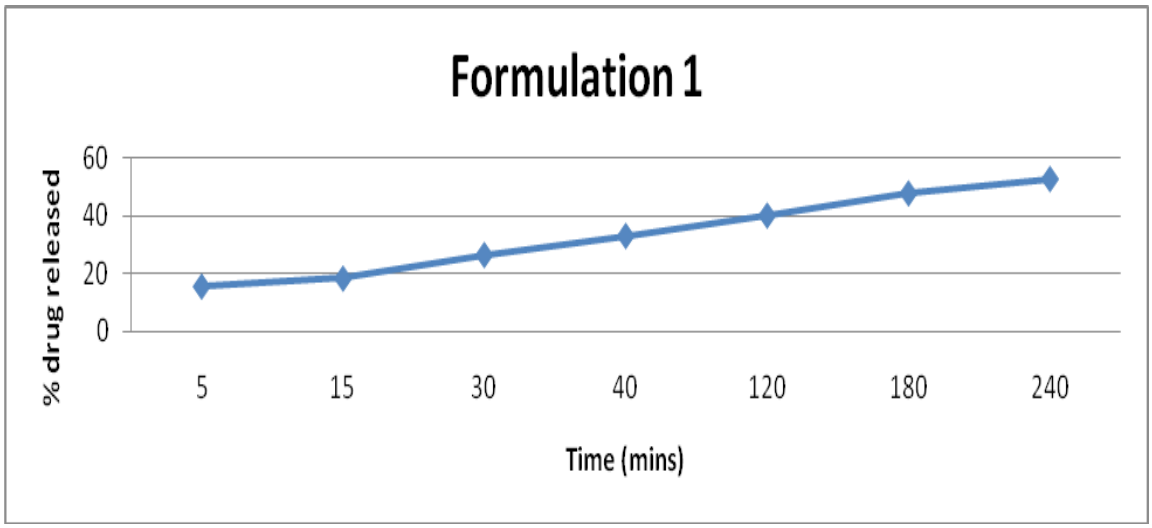


Table20: **Data showing release profile Formulation 2 of through dialysis membrane from phase transition system of chitosan hydrochloride.**

Time(mins)	Absorbance(nm)	conc.(µg/ml)	% release
5	0.0844	1.688	8.44
15	0.1427	2.854	14.27
30	0.1990	3.98	19.90
40	0.2812	5.624	28.12
120	0.310	6.20	31.0
180	0.3729	7.458	37.29
240	0.4196	8.392	41.96

Figure 20 :Graph showing the release profile of formulation 2 through dialysis membrane from phase transition system of chitosan hydrochloride

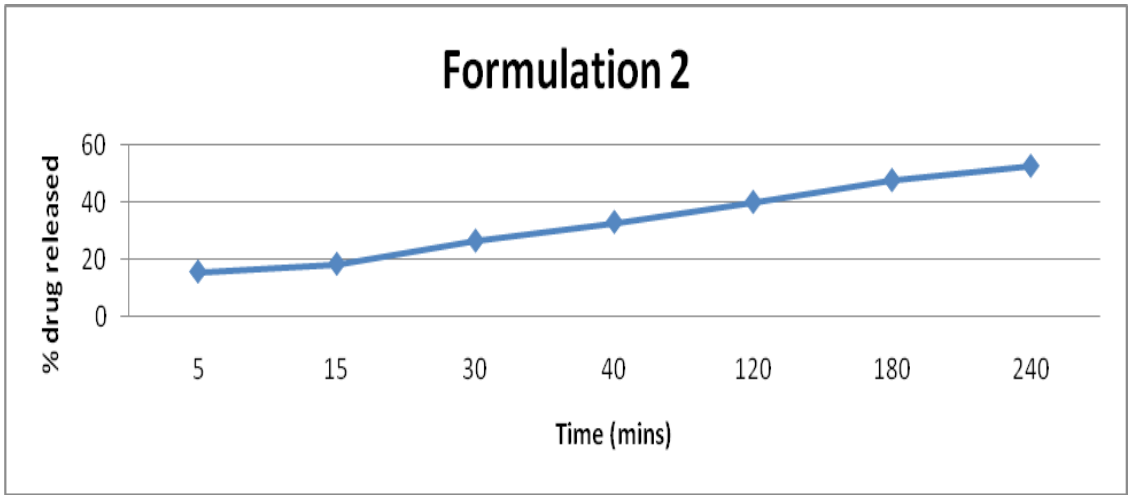


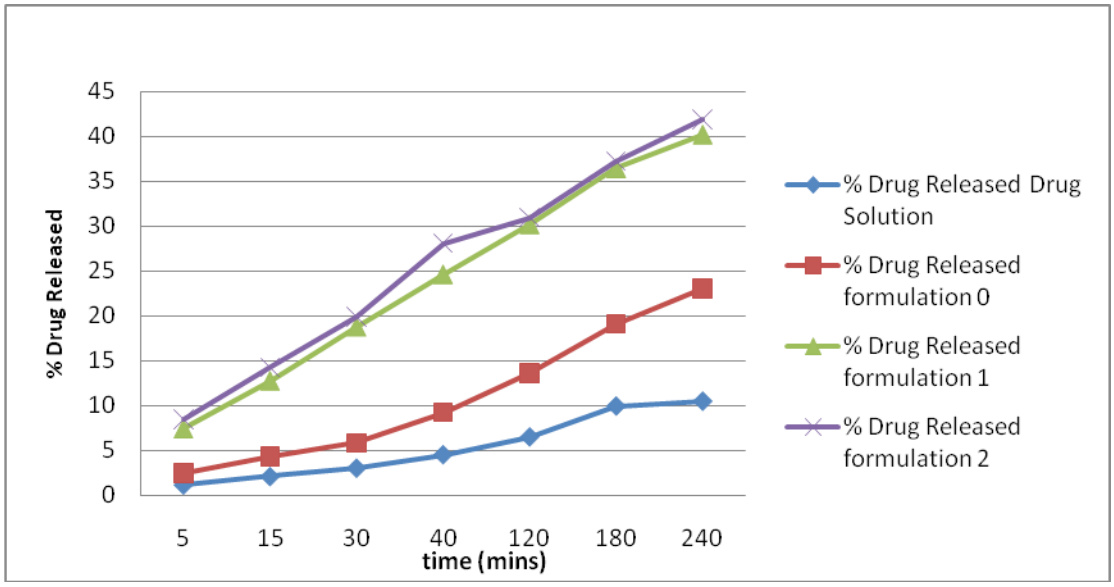
Table 21: Data showing release profile of drug through dialysis membrane from

phase transition system of Chitosan Hydrochloride

Time (min)	Cumulative % drug released			
	Drug Solution	Formulation 0	Formulation 1	Formulation 2
Time(mins)	1.12	2.46	7.51	8.44
Time(mins)	2.06	4.36	12.82	14.27
Time(mins)	3.0	5.86	18.84	19.90
Time(mins)	4.5	9.25	24.66	28.12
Time(mins)	6.48	13.64	30.23	31.0
Time(mins)	9.93	19.13	36.46	37.29
Time(mins)	10.47	23.07	40.20	41.96

Figure 21:

Release profile of drug through dialysis membrane from phase transition system of Chitosan Hydrochloride



***In vitro* permeation study**

Table 22 : Permeation profile of plain drug solution through porcine nasal membrane

Time(mins)	Absorbance(nm)	conc.(µg/ml)	% drug permeated
5	0.0212	0.424	2.12
15	0.0348	0.696	3.48
30	0.0483	0.966	4.83
40	0.0772	1.544	7.72
120	0.1047	20.94	10.47
180	0.1368	2.736	13.68
240	0.1546	3.092	15.46

Figure 22 :

Graph showing the permeation profile of plain drug solution through porcine nasal membrane.

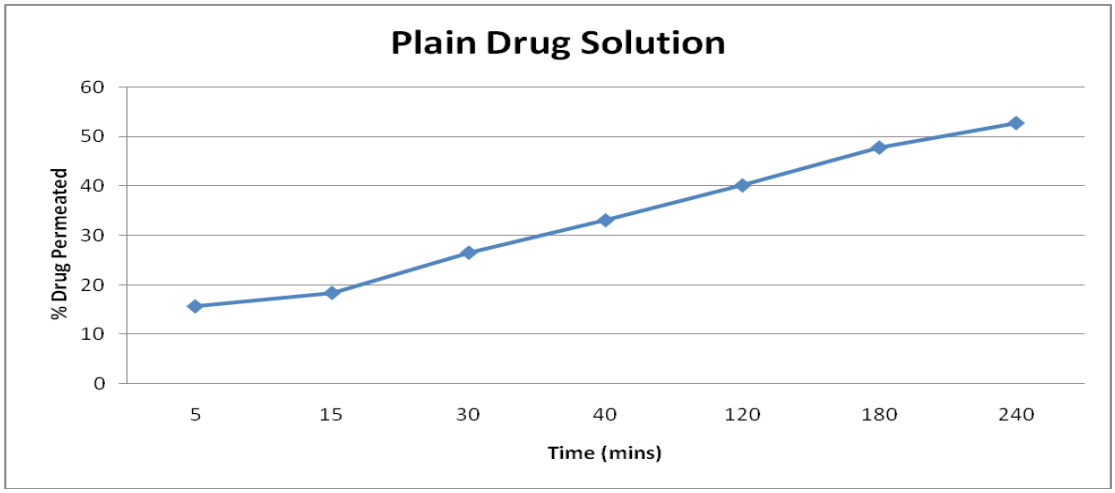


Table 23 : Permeation profile of Formulation 0 through porcine nasal membrane

Time(mins)	Absorbance(nm)	conc.(µg/ml)	% drug permeated
5	0.0540	1.08	5.40
15	0.0652	1.304	6.52
30	0.1128	2.256	11.28
40	0.1634	3.268	16.34
120	0.2132	4.264	21.
180	0.2712	5.424	27.12
240	0.3156	6.312	31.56

Figure 23 :

Graph showing the permeation profile of formulation0 through porcine nasal membrane

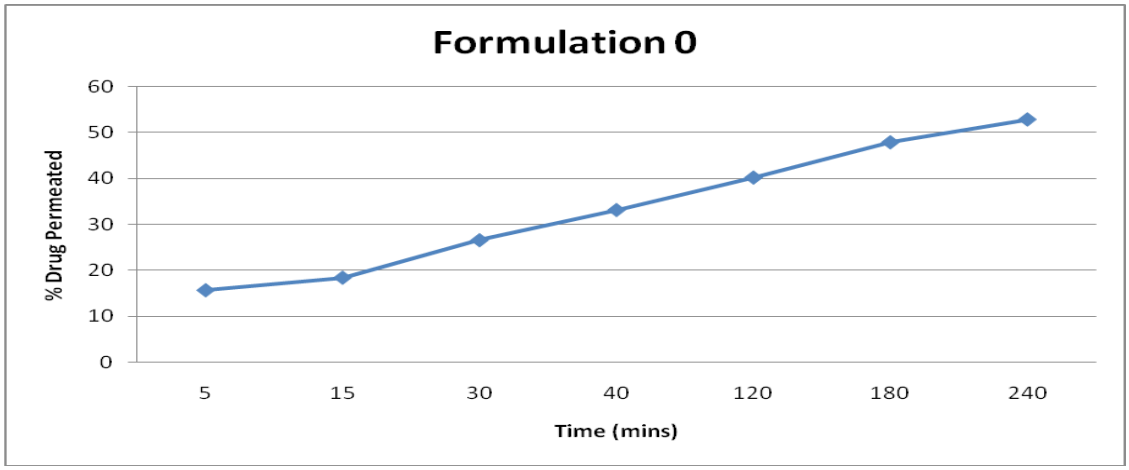


Table 24 :
Permeation profile of Formulation 1 through porcine nasal membrane

Time(mins)	Absorbance(nm)	conc.(µg/ml)	%drug permeated
5	0.1423	2.846	14.23
15	0.1754	3.508	17.54
30	0.2442	4.884	24.42
40	0.3132	6.264	31.32
120	0.3854	7.708	38.54
180	0.4658	9.316	46.58
240	0.5126	10.252	51.26

Fig 24 :

Graph showing the permeation profile of formulation1 through porcine nasal membrane

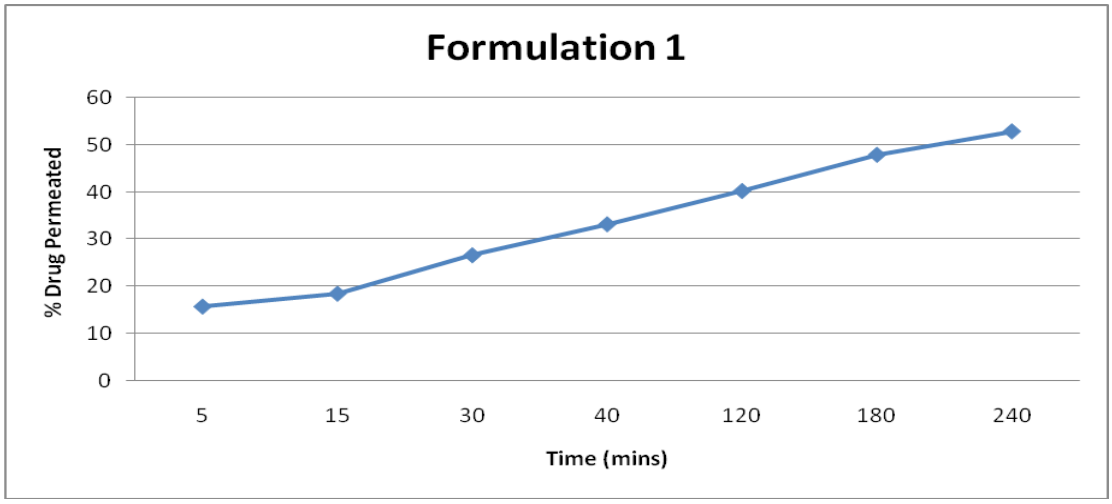


Table 25 :
Permeation profile of Formulation 2 through porcine nasal membrane

Time(mins)	Absorbance(nm)	conc.(µg/ml)	%drug permeated
5	0.1570	3.14	15.70
15	0.1842	3.684	18.42
30	0.2656	4.884	26.56
40	0.3312	6.264	33.12
120	0.4016	80032	40.16
180	0.4782	9.564	47.82
240	0.5278	10.556	52.78

Fig 25:
Graph showing the permeation profile of formulation2 through porcine nasal membrane

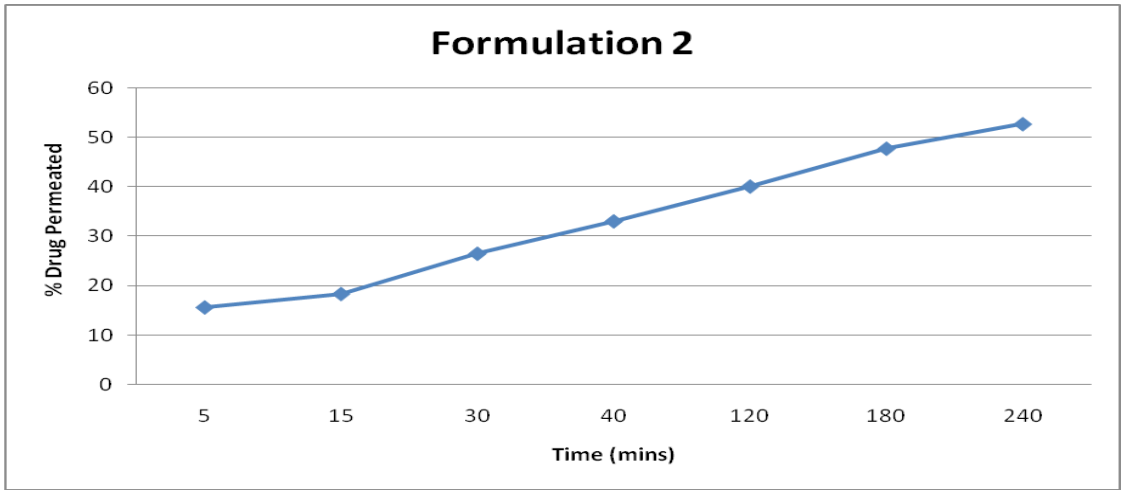


Table 26 : cumulative % of Drug permeated through porcine nasal membrane

Time (min)	Drug Solution	Formulation 0	Formulation 1	Formulation 2
5	2.12	5.40	14.23	15.70
15	3.48	6.52	17.54	18.42
30	4.83	11.28	24.42	26.56
60	7.72	16.34	31.32	33.12
120	10.47	21.32	38.54	40.16
180	13.68	27.12	46.58	47.82
240	15.46	31.56	51.26	52.78

Figure 26 : cumulative % of drug permeated through porcine nasal membrane

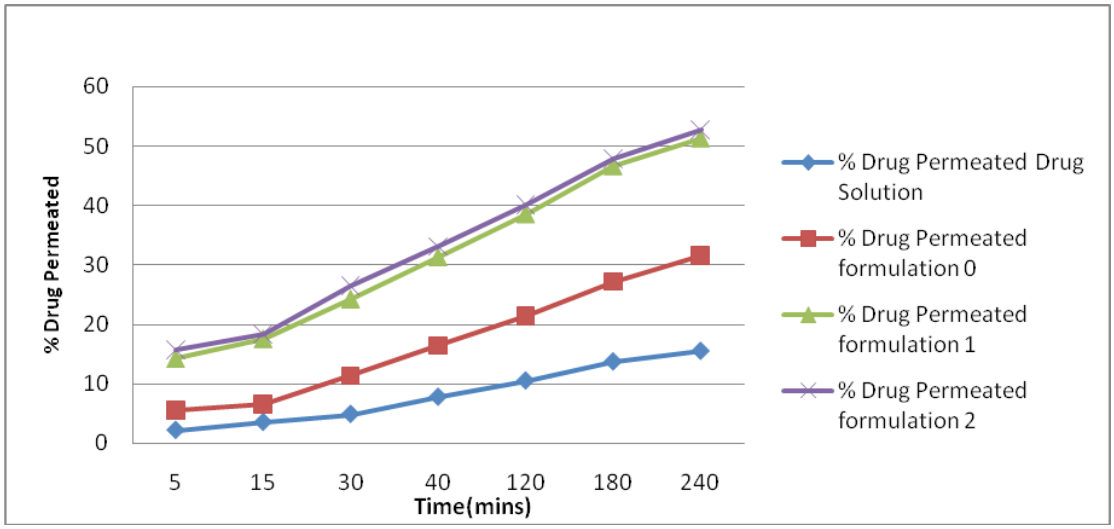


Table 27 : **Permeability Coefficient of formulations**

Formulations	**Membrane used**	**Permeability coefficient (10 $^{-6}$cm/sec)**
Drug solution	Nasal	3.68
0	Nasal	7.19
1	Nasal	9.17
2	Nasal	9.27

Drug content

Table 27 : **Drug content of formulated phase transition system**

Sr. No	Formulations	Drug content (%)
1	0	99.82
2	1	98.61
3	2	98.14

Stability Studies

Table 28 : Evaluation of formulation 1 after different time interval at different temperature

Temperature / Parameters Evaluated		Storage			
		0 month	1 month	2 month	3 month
10 ^{0}C ± 2 ^{0}C 75 % ± 5 % Rh	Drug Content (%)	98.61	98.60	98.60	98.59
	Viscosity (P)	1.05	1.05	1.05	1.05
	Gelation temperature (^{0}C)	35-37	35-37	35-37	35-37
25 ^{0}C ± 2 ^{0}C 75 % ± 5 % Rh	Drug Content (%)	98.61	98.60	98.59	98.59
	Viscosity (P)	2.40	2.40	2.40	2.40
	Gelation temperature(^{0}C)	35-37	35-37	35-37	35-37
40 ^{0}C ± 2 ^{0}C 75 % ± 5 % Rh	Drug Content (%)	98.61	98.59	98.58	98.58
	Viscosity (P)	15.85	15.85	15.85	15.85

Table 29 : Evaluation of formulation 2 after different time interval at different temperature

Temperature / Parameters Evaluated		Storage			
		0 month	1 month	2 month	3 month
10 ^{0}C ± 2 ^{0}C 75 % ± 5 % Rh	Drug Content (%)	98.14	98.12	98.13	98.13
	Viscosity (P)	2.20	2.20	2.20	2.20
	Gelation temperature (^{0}C)	34-37	34-37	34-37	34-37
25 ^{0}C ± 2 ^{0}C 75 % ± 5 % Rh	Drug Content (%)	98.14	98.13	98.12	98.12
	Viscosity (P)	3.46	3.46	3.46	3.46
	Gelation temperature(^{0}C)	34-37	34-37	34-37	34-37
40 ^{0}C ± 2 ^{0}C 75 % ± 5 % Rh	Drug Content (%)	98.14	98.14	98.13	98.12
	Viscosity (P)	17.20	17.20	17.20	17.20

Formulations showed good stability with no change in drug content, viscosity and gelation temperature after stability study of 3 months.

DISCUSSION

The developed formulations were evaluated for Gelation Temperature, viscosity, Mucoadhesion, duration of mucoadhesion, *in vitro* release, *in vitro* permeation, & percent drug content.

Gelation Temperature Gelation temperature observed for formulation 1 was in the range of 35-37 ^{0}C and that of formulation 2 was in the range of 34-37 ^{0}C. Gelation temperature is the temperature at which liquid phase makes transition to gel. Gelation temperature range suitable would be 30-36 ^{0}C.

If the gelation temperature is lower than 30 ^{0}C, gelation occurs at room temperature leading to difficulty in administering .If the gelation temperature is higher than 36 ^{0}C, it will leak out from the nasal cavity. Thus the suitable gelation temperature is 30-36 ^{0}C, to be a liquid form at room temperature and to form a gel phase in the nasal cavity.

Viscosity Measurements

The values of viscosity in poise for formulation 1 at 10^{0}C (1.05), 25^{0}C (2.40), 40^{0}C (15.85) and that of formulation 2 at 10^0(2.20), 25^0(3.46) and 40^0(17.20) were observed. The rise in viscosity with rise in temperature may be because of the formation of gel as chitosan along with glycerophosphate on heating shows phase transition. The addition of glycerophosphate salt to chitosan aqueous solution directly modulates electrostatic and hydrophobic interactions (dehydration of chitosan with increase in temperature leads to increased hydrophobic interaction between the molecules) and hydrogen bonding between chitosan chains, which are the main molecular forces involved in the gel formation. This study supported by Chenite et al

Rheological behavior

Rheological characteristics of formulation 1 and 2 was studied using Brookfield CAP Viscometer and rheograms prove that both the formulations exhibited pseudoplastic flow as evidence by shear thinning with the increase in speed of spindle.

Mucoadhesion study

The formulations 1 and 2 were subjected to mucoadhesion study & the mucoadhesive force of fromulation 1 was observed to be 24.63 x 10^2 dynes/cm^2 and that of formulation 2 was found to be 32.39 x 10^2 dynes/cm^2 . The mucoadhesive force is an important physicochemical parameter for phase transition system of nasal formulations since it prevent the gels from nasal clearance and increase the residence time in the nasal cavity.

The reinforcement of the mucoadhesive forces of the nasal phase transition system by the use of mucoadhesive polymers could be explained by the fact that secondary bond forming groups (hydroxyl, ether, oxygen and amine) are the principle source of mucoadhesion. This study is supported by Elhady et al. The bioadhesive force is known to depend on the nature and the concentration of bioadhesive polymers. With the increase in polymer concentration mucoadhesion was found to increase. The stronger the bioadhesive force more is the residence time in nasal cavity. But if the bioadhesive force is too excessive the gel can damage nasal mucous membrane.

Duration of mucoadhesion

Duration of mucoadhesion carried out by two different method shows the time that the formulation can remain in contact with the membrane. The duration by method 2, for formulation 1 was 4.05 hrs. and for formulation 2 was 4.30 hrs. was found shorter as compared to agar plate method i. e. method 1 (movement of formulation less than 3cm even after 4 hrs). This may be because of the higher stress applied on the formulation in case of method 2 as compared to method 1.

***In vitro* release study**

Formulations were subjected to in- vitro release studies using synthetic cellulose nitrate membrane, at 37^0C. Phosphate buffer saline 7.4 was selected as diffusion membrane. The % drug release by plain drug solution (10.47), formulation 0 (23.07), formulation 1 (40.20) and formulation 2 (41.96) was calculated for 4 hours and is mentioned in the table.

***In vitro* permeation study**

Permeability coefficient of drug solution through nasal membrane was 3.68 x 10^{-6} cm/sec. This may be because nasal membrane is comparatively very thin and may be because it contains large number of pores, this is supported by the study of M. D. Chavanpatil.

Permeability coefficient of drug solubilised by HP β cyclodextrin (7.19x10^{-6} cm/sec) was even greater than plain drug therefore HP β cyclodextrin serves two purposes, one increase the solubility of drug and second improves the permeability of the drug. The formulation 1 and 2 show extremely significant ($P<0.01$) change in permeability coefficient (9.17x10^{-6} cm/sec for formulation 1 and 9.27x10^{-6} cm/sec for CHT 2) than plain drug, this may be because the formulation 1 and formulation 2 contain HP β cyclodextrin along with chitosan hydrochloride, which shows synergistic effect on the permeation of drug. Chitosan hydrochloride open up the tight junction and HP β cyclodextrin extracts the phospholipids and proteins from membrane by forming a new inclusion compartment in the aqueous phase. This statement is supported by Shayun Yu and Zhang Q.

Drug Content

The percent drug content of formulation 0 was found to be 99.82, formulation 1 found to be 98.61 and that of formulation 2 was 98.14. The drug content of phase transition system is shown in table.

The use of chitosan hydrochloride in phase transition system is substantiated by the property of its aqueous solution along with glycerophosphate to transform into hydrogel when the temperature is raised to physiological conditions demonstrated by viscosity measurement.

CH. VIII.

SUMMARY AND CONCLUSION

Nasal delivery is a feasible alternative to oral or parenteral administration for some drugs because of the high permeability of the nasal epithelium rapid drug absorption across this membrane and avoidance of hepatic first pass metabolism.

The nasal dosage form includes solution, sprays, microspheres, gels and liposomes. Although solutions are easy to use they achieve a poor bioavailability, due to large mucocilliary clearance. It had been demonstrated that a significant improvement in bioavailability would be achieved if the nasal residence time of the drug would be increased. From the point of view of patient acceptability a liquid dosage form that can be administered easily and can adhere to the nasal mucosa for extended period with fast onset of action is ideal.

Drug delivery system based on the concepts of phase transition system should provide these properties, such delivery system show phase transition due to a change in specific physicochemical properties (pH, Temperature, Ionic content) in their environments.

In the present work, attempt was made to develop phase transition system with mucoadhesive properties that can be easily administered as drops and attains semisolid properties at physiological temperature at the site of absorption, improves bioavailability giving fast onset of action with reduced dosing compared to oral drug delivery.

The formulation 1 and 2 containing 1% chitosan hydrochloride and 2 % chitosan hydrochloride respectively were subjected to various evaluation parameters.

For both the formulations marked increase in viscosity was observed when the temperature was raised upto 37^0C. From the duration of mucoadhesion study it was found that both the formulations can adhere to the nasal mucosa at least for a period of 4 hours. No significant differences in permeability coefficient were observed when subjected to *in vitro* permeation study.

So, from the above observation the nasal permeation and the retention time of drug increases by the addition of increasing percentage of chitosan in both the formulation,and formulation 2 is considered as best formulation .

CH. IX.

FUTURE SCOPE

- *In-vivo* study to determine pharmacokinetic and pharmacodynamic parameters.
- Gel formation study in nasal cavity.
- Drug clearance study from nasal cavity.

CH.X.

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